BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS OF OBESITY, HIGH FAT DIET, AND PROLONGED FASTING IN FREE-RANGING POLAR BEARS

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by

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ABSTRACT

The principle objective of this investigation was to develop an understanding of the biochemical and physiological response of free-ranging adult polar bears (*Ursus maritimus*) to prolonged fasting. A body condition index was developed from two measures, total body mass and straight-line body length, and was used as a covariate in the analyses of all other data. Protein and amino acid catabolism and urea synthesis were significantly lower in fasting bears when compared to feeding bears, and in fat bears when compared to lean bears. The inference from these results is that the energy metabolism in both states (fasting and fat) is one in which lipid is the predominant fuel for energy and nitrogen is conserved. Nutritional state (feeding versus fasting) had no significant effect on the plasma concentrations of non-esterified fatty acid, glycerol, and ketone bodies, or on the plasma ratio of acyl-carnitine to free carnitine. Furthermore, acetoacetate concentration was below the level of detection (< 196 μmol/L) in all bears, and β-hydroxybutyrate concentration never exceeded 291 μmol/L. These results suggest polar bears are able to regulate closely the synthesis, release, and use of lipid metabolites without significant alteration in their plasma concentrations. Fasting polar bears showed no evidence of essential fatty acid (EFA) deficiency; the proportions of the diet-derived EFA linoleic (18:2ω6) and α-linolenic (18:3ω3) acids in the plasma and adipose tissue of fasting polar bears were greater than that in feeding polar bears. Plasma triiodothyronine concentrations and rectal temperatures were lower in fasting bears captured during summer-fall than in feeding bears, which suggests metabolic rates were decreased during fasting to conserve body fuels. Liver glycogen concentrations were found to be higher in fasting polar bears than in feeding bears. Furthermore, the results from intravenous administration of glucose (glucose tolerance test) to polar bears indicated the rates of insulin secretion and clearance in polar bears were slow relative to rates reported for other mammals. The inference from these results is that polar bears are not as dependent on glucose for energy as are other mammals and, as a consequence, are more lax in regulating their body glucose stores.

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LIST OF ABBREVIATIONS

AC	Acyl-carnitine
ALC	Lipid content of adipose tissue
AWC	Water content of adipose tissue
BCI	Body Condition Index
BLC	Body lipid content
BMR	Basal metabolic rate
BRANCH	Branched-chain fatty acid
BWC	Body water content
DBI	Double bond index
DP	Desaturase product
E:NE ratio	Ratio of essential fatty acids and products to non-essential fatty acids and products
EFA	Essential fatty acid
EP	Elongase product
FC	Free carnitine
$FI_{chemical}$	Fatness Index: determined from the results of whole body chemical composition
FI_{dd}	Fatness Index: determined from the results of deuterium dilution
$FI_{dissect}$	Fatness Index: determined from the results of gross tissue dissection
FM	Fat mass
HDL	High density lipoprotein
I/G	Insulin to glucagon molar ratio
LBM	Lean body mass
LDL	Low density lipoprotein
MCL	Mean chain length in carbon atoms
MONO	Monoenoic fatty acid
NEFA	Non-esterified fatty acid
ODD	Odd-chain fatty acid
P	Product
PCA	Perchloric acid
PUFA	Polyunsaturated fatty acid
QI	Quetelet Index
R_a	Rate of appearance
R_c	Rate of clearance
SAT	Saturated fatty acid
SLBL	Straight-line body length
$T_{½}$	Half-life

T3	**Triiodothyronine**
T4	**Thyroxine**
TBM	**Total body mass**
TRI:TET ratio	**Ratio of the triene, 20:3ω9, to the tetraene, 20:4ω6**

1. GENERAL INTRODUCTION

1.1 Similar scenarios, different outcomes

Of all of her 29 years, 1995 was promising to be the best for Janice. Only one year earlier, she had been hired as a graphic artist by an innovative and highly successful group of Ottawa-based, computer programmers. Although she had only a vague understanding of the world of computer programming, Janice did have a remarkable ability to express her artistic talent with a keyboard, mouse, and monitor. Thus, in only six short months, 'home pages designed by Janice' formed the web-site frontispiece for many an Ottawa company, and were in demand by many more. Where sudden success and a soaring salary unravel the day-to-day habits of most people, Janice remained unchanged in much of her lifestyle and only acknowledged her newly-acquired fame and fortune with a mixture of disbelief and modest amusement. Nevertheless, the combination of frequent meetings with new clients and a simmering attraction for a co-worker had instilled in Janice a burning desire to change one aspect of her life. Janice wanted to lose weight, and more strongly now than at any other time in her life.

On December 1st, 1994, Janice weighed 93.2 kilograms on her doctor's scales, but was otherwise in good health. Nevertheless, from scanning the Body Mass Index chart above the weigh scale, Janice had been quick to determine that she fell into the category of individuals known as 'the obese', her body weight being approximately 32 kilograms above the ideal body weight for her height. Appalled at her physical designation and attributing it to years of consuming junk food, Janice returned home following her appointment and emptied her cupboards and refrigerator of all foods. Melba toast, rice cakes, green food, and low-fat yoghurt were to form the bulk of her new diet, and a 45 minute morning session on the Stair-Master was to become a daily ritual. Her goal, to lose 35 kilograms prior to her 30th birthday.

Thirteen weeks later, and with still more than one week remaining until her birthday, Janice weighed 57 kilograms. An intense work schedule, a very low caloric diet, the morning sessions on the Stair-Master, and perhaps the more than occasional popping of decongestant tablets had helped to transform Janice. And yet, despite her obvious elation and assertions of "I've never felt better !", Janice's doctor was somewhat guarded in sharing the joy. In fact, she was quite troubled by the irregular heartbeat she had detected when listening to Janice's chest with her stethoscope. Although irregular heartbeats most often prove to be of little significance in many people, there was a dim recollection in the doctor's mind that an irregular heartbeat coupled with rapid and large weight loss warned of something more serious. She would need to refer to her medical reference books on this, but in the meantime she decided to book Janice for an electrocardiographic assessment as soon as possible at the hospital's cardiology centre.

Janice never did attend her appointment at the cardiology centre. In fact, only two hours after leaving her doctor, she collapsed while attending a business meeting. Attempts at cardiopulmonary recuscitation and a rapid response by the paramedics were futile. Janice was declared dead upon arrival at the hospital. Two days later, the pathologist's report was to explain the cause of death as a result of myocardial degeneration, the wasting away of the muscle cells forming the heart. Presumably, the months of extreme dieting had stripped away from Janice the proteins that were essential to the integrity of her heart, and consequently her life, so as only to provide her with the fuel that she needed to meet her daily schedule. In essence, her body had simply self-destructed.

At the same moment that Janice collapsed, X09111 also collapsed approximately 1700 kilometers northwest of Ottawa on the southwest coast of Hudson Bay. At 29 years of age, X09111 was one of the oldest polar bears (*Ursus maritimus*) listed in the data records of the government wildlife service. And this was to be the fourteenth time she had been captured by the biologists. As X09111 slowly succumbed to the effects of the immobilizing drug, her two newly-born cubs clung to her, their island of safety on a hostile sea of snow and ice.

Nine months earlier, X09111 had been captured minutes after swimming ashore from one of the last remaining pans of melting sea-ice on Hudson Bay. The biologists had guessed from her

rotund appearance, and the fact that she wasn't accompanied by other bears, that she might be pregnant. Before releasing X09111, they fitted her with a radio-collar, collected some of her blood, and measured her body dimensions in various ways. To the biologists, the most remarkable feature of X09111 was her enormous body weight, exactly 414 kilograms, of which they had no doubt that at least half was fat.

Now, in March 1995, the biologists' guess at the pregnant state of X09111 no longer remained in question, being unmistakably confirmed by the presence of two healthy male cubs. Furthermore, X09111 had lost a whopping 218 kilograms during the past nine months, her physical stature now only a shadow of its former self. Nevertheless, this was not the first time that X09111 had lost almost half her body weight in less than a year. At three previous times, X09111 had lost similar amounts of weight during fall and winter, only to turn up again during the spring with a couple of cubs in tow.

The scenarios are similar. Both stories tell of the loss of a large amount of body weight. The outcomes, however, are different. Where weight loss leads to death in one story, it is a recurring feature of life in the other.

1.2 An objective and a context

How is it that the process of weight loss can be fatal in one mammal, and yet appearingly benign in another? This question is a central theme of this thesis. In the case of humans, there is much known about their biochemical and physiological response to food deprivation. In the case of polar bears, there is little known to explain how these animals are able to survive extreme lengths of total food deprivation without any apparent consequence to their health.

The primary objective of this investigation was to begin to develop some understanding of the biochemical and physiological response of free-ranging adult polar bears to prolonged fasting. Free-ranging bears were studied rather than captive bears because it seemed unlikely that the results taken from a small number of captive polar bears reared within the artificial environment of a zoo or marine park could ever be accepted to represent the species as a whole. This is not to say, however,

that future studies of captive polar bears could not be used to build on the information obtained in this study of free-ranging polar bears. Adult polar bears were studied rather than younger bears because of the possibility that biochemical and physiological mechanisms, particularly those that facilitate the adaptive response to prolonged fasting, might not be fully developed in the younger bears.

The interpretation of results from this study frequently was done in a comparative manner, and most often with humans as the species for comparison. This was for two reasons. First, details regarding the biochemical and physiological response to food deprivation in mammals are probably most numerous, and possibly most complete, for humans. Thus, the knowledge developed from studies of humans seemed a logical basis from which to compare and interpret the results obtained from polar bears. Second, beyond the effects of prolonged fasting, there are effects of other dietary features that appear to be profoundly different in humans and polar bears. Thus, in humans, obesity and consumption of foods that are high in fat content are associated with a suite of metabolic diseases, e.g., coronary heart disease, hyperglycemia, insulin resistance, and type II diabetes mellitus. In contrast, obesity and the consumption of foods high in fat appear to be critical factors that enable polar bears to withstand prolonged periods without food, and do not appear to impact upon the health of polar bears as occurs in humans. Recognizing these species differences, it would appear that points of departure between humans and polar bears in their biochemical and physiological response to food deprivation may be areas where further comparative investigation could provide new insight into the pathogenesis, and perhaps the treatment, of some of the metabolic diseases in humans.

1.3 The dietary features of polar bears

Polar bears typically consume large quantities of seal fat during the spring, whereas the remainder of the year is characterized by intermittent fasts of variable duration ranging from days to months. From April to July, polar bears prey intensively on fat, young-of-the-year, ringed seals (*Phoca hispida*) (Stirling and Archibald 1977, Smith 1980, Hammill and Smith 1991). After killing

a seal, a polar bear often will eat the fat of the seal and leave the rest of the carcass (Stirling 1974). As a result of the large fat content of young weaned seals (Stirling and McEwan 1975), coupled with the exceptional ability of polar bears to digest large quantities of fat (Best 1984), polar bears become fat between spring and summer (Ramsay and Stirling 1988). In Hudson Bay, seals become unavailable to most polar bears during the open-water season of summer and fall, and bears become inactive and fast for up to several months at a time (Nelson et al. 1983, Derocher et al. 1990, Ramsay et al. 1991). When sea-ice re-forms during late fall, seals become accessible again and most polar bears return to the ice to resume hunting (Stirling 1974, Latour 1981, Derocher and Stirling 1990). Nevertheless, inclement weather and more wary seals (relative to the young seals captured during spring) are believed to reduce the frequency of prey capture from that observed during spring (Stirling and Øritsland 1995). In contrast to other adult bears, pregnant bears remain on land in maternity dens throughout winter and emerge from their dens with one to three cubs, in March, after 8 to 9 months of continuous fasting (Ramsay and Stirling 1982, Watts and Hansen 1987). Together, the circannual dietary features of polar bears suggest that survival of the species is contingent upon an ability to closely regulate their accretion of adipose tissue and their metabolism of stored fuels (lipid, protein, and glucose).

1.4 The thesis

The body of this thesis is an integrated collection of five parts. The first part (Chapter 2) presents a body condition index (BCI) that was developed during this study to compare body condition among individual polar bears regardless of their sex, age, reproductive class, geographical population, or season-of-capture. The second part (Chapter 3) considers the process of protein-sparing in polar bears by examining the effect that nutritional state (feeding versus fasting), sex (female versus male), and body condition (lean versus fat) have upon the concentrations of proteins, enzymes, free amino acids, and nitrogen end-products in plasma, and upon the activities and concentrations of enzymes and metabolites in liver that are involved in the ornithine-urea cycle and in intermediary metabolism. The third part (Chapter 4) examines the effect that nutritional state, sex,

and body condition have upon the concentrations of lipids, lipid precursors and metabolites, total carnitine, and carnitine fractions in plasma, and upon the kinetics of plasma glycerol in polar bears. The fourth part (Chapter 5) considers the effect that diet and prolonged fasting have upon the fatty acid composition of polar and black bears (*Ursus americanus*) by examining the fatty acid composition of three lipid fractions (triacylglycerol, non-esterified fatty acid, and phospholipid) in plasma, and one lipid fraction in adipose tissue (triacylglycerol), in feeding and fasting bears. The fifth part (Chapter 6) considers the regulation and metabolism of carbohydrate in polar bears by examining the effect that nutritional state, sex, and body condition have upon their concentrations of glucose, cortisol, triiodothyronine, thyroxine, insulin, and glucagon in plasma, as well as upon the change in their plasma insulin and glucagon concentrations following intravenous administration of glucose (glucose tolerance test).

2. THE DEVELOPMENT AND ASSESSMENT OF A BODY CONDITION INDEX FOR FREE-RANGING POLAR BEARS

2.1 Introduction

Body condition can be defined as a measure of the abundance of potential energy stored in the body tissues (primarily fat and skeletal muscle) of an animal relative to its body size. As a biological variable, body condition has been important for monitoring long-term trends in the fluctuation of food availability in wild populations (Thomas et al. 1982, Costa et al. 1989, Hellgren et al. 1993, Stirling et al. 1999) and for addressing ecological issues (Messier and Crête 1984, Ryg et al. 1990, Atkinson and Ramsay 1995). Body condition can be also an important variable in physiological and biochemical investigations because of its potentially confounding effects on other parameters (Verrillo et al. 1988, Lewis et al. 1990, Vansant et al. 1991).

Body condition in polar bears (*Ursus maritimus*) has been measured or estimated by morphometric measurements, chemical analysis of the whole carcass, measurement of fat in bone marrow and among muscles, bioelectrical impedance, and deuterium dilution (Watts and Hansen 1987, Cattet 1988, Cattet 1990, Arnould and Ramsay 1994, Farley and Robbins 1994, Atkinson and Ramsay 1995, Atkinson et al. 1996a). Morphometric measurements have been imprecise, in large part because of variation in the measurement of linear dimensions and the compounding of the resulting error by morphometric prediction equations (Cattet 1990, Cattet et al. 1997). Furthermore, the accuracy and precision of these prediction equations have been found to be significantly affected by sex, age, reproductive class, and geographical population. Chemical analysis of the whole carcass and the measurement of fat in bone marrow and among muscles are time-consuming techniques that require dead bears and laboratory analysis. Bioelectrical impedance and deuterium dilution have been advocated as the two most useful methods available to determine the body composition and condition of living bears (Farley and Robbins 1994, Hilderbrand et al. 1998) and both techniques

have been applied to polar bears (Farley and Robbins 1994, Atkinson and Ramsay 1995, Atkinson et al. 1996a). Nevertheless, the relationships between chemical composition and electrical conductance or deuterium dilution have not been determined in this species.

This investigation had two objectives. The first was to develop a body condition index (BCI) for free-ranging polar bears which could be easily measured and used to compare individual animals regardless of sex, age, reproductive class, geographical population, or season-of-capture. The second was to evaluate the use of the BCI by comparing it with two other indices that have been used in recent years to measure body condition in free-ranging polar bears.

2.2 Materials and methods

The sample population comprised 1072 free-ranging polar bears that represented three geographical populations, both sexes, and all age and reproductive classes (Table 2.1). All bears were captured as part of ongoing studies from 1984 to 1996 and details of capture and handling procedures, measurements, geographical populations, and designation of sex, age, and reproductive class, have been described previously in Kolenosky et al. (1989) and Cattet et al. (1997).

The body condition index (BCI) was developed from two measures, total body mass (TBM) and straight-line body length (SLBL; the straight-line distance from the tip of the nose to the end of the last tail vertebra), that were routinely recorded during the handling of captured polar bears. The mass and length data for 1072 polar bears were transformed to natural logarithms and regression analysis was used to describe the curvilinear relationship between the dependent variable, TBM, and the independent variable, SLBL, separately for female (n = 494) and male (n = 578) polar bears (SPSS® 6.1 for Windows®, SPSS Inc., Chicago, Illinois, USA). Slopes and intercepts for sex-specific regression equations were then compared using large sample Z-tests for parallelism and common intercept, respectively (Kleinbaum and Kupper 1978).

Table 2.1. Description of sample polar bear population by sex, age, reproductive class, and geographical location.[a]

Sex, age, and reproductive class	Geographical Population WH	SH	LS	Combined populations
solitary adult female	44	27	19	90
adult female with cubs	150	56	26	232
juvenile females	109	56	7	172
adult males	176	174	56	406
juvenile males	95	59	18	172
Population totals	574	372	126	1072

[a] Populations are from western Hudson Bay (WH), southern Hudson Bay (SH), and Lancaster Sound (LS). The LS population was previously named the Parry Channel (PC) population in Cattet et al. (1997).

The BCI value of each bear was equivalent to its standardized residual, which was determined from the regression of TBM on SLBL and calculated as:

$$BCI = d_i / S \qquad \text{(Eqn. 2.1)}$$

Where d_i is the deviation or residual of the observed TBM above or below the predicted TBM for the ith bear, and S is the standard deviation of all the residuals calculated as $[1/(n - k + 1) \times \sum d_i^2]^{-½}$ where n is the number of bears, k is the number of independent variables, and $\sum d_i^2$ is the sum of the squared residuals for all bears (Kleinbaum and Kupper 1978, Zar 1996). The BCI values for all bears were graphically analyzed to determine how well the data conformed with a normal distribution. Pearson correlation analysis was used to determine if the association between the BCI and SLBL was significant ($p < 0.05$).

The relative contribution of the BCI and SLBL to explaining variation in the mass of dissectable fat, skeletal muscle, potential energy tissue (fat + skeletal muscle), and structural tissue (skin and fur + bone + viscera) in 31 polar bears was determined using stepwise regression analysis (Kleinbaum and Kupper 1978). Details regarding the collection and dissection of these bears have been described previously by Cattet (1990). The viscera were comprised of all organs contained within the thoracic and abdominal cavities.

Four nomograms were constructed to enable rapid determination of BCI values for individual polar bears. Values for SLBL ranging from 60 to 70 cm, 70 to 130 cm, 130 to 190 cm, and 190 to 270 cm were placed along the abscissae of the nomograms, whereas TBM values ranging from 3 to 18 kg, 5 to 115 kg, 30 to 390 kg, and 100 to 1100 kg were placed along the ordinates. Equations for BCI lines with values of -2.0, -1.5, -1.0, -0.5, 0, +0.5, +1.0, +1.5, and +2.0 were calculated by regression analyses of the SLBL and TBM data for 1072 bears which were subdivided into 9 groups (group size ranged from 26 to 199 animals) such that within each group the BCI values were similar among bears, i.e., BCI values in the first group were > -2.25 and ≤ -1.75, BCI values in the second group were > -1.75 and ≤ -1.25, ..., BCI values in the ninth group were > +1.75 and ≤

+2.25. The different BCI lines were then plotted on each of the nomograms to allow an estimate of the BCI to be made at the point of intersection between paired values for SLBL and TBM.

To evaluate the use of the BCI in estimating the body condition of different bears captured during different months (i.e., cross-sectional comparison), the BCI was compared with the Quetelet Index (QI) in estimating the body condition of 420 polar bears. The QI (also known as the Body Mass Index) was originally developed to assess obesity in humans (Ganong 1995). However, it has been applied recently to polar bears and, in this species, is calculated as TBM ÷ $SLBL^2$ (kg/m^2) (Stirling et al. 1999).

To evaluate the use of the BCI in estimating the change in body condition of the same bears captured at two different times (i.e., longitudinal comparison), the BCI was compared with the Fatness Index in estimating the change in the body condition of 20 fasting polar bears captured at two different times between July and October. The Fatness Index was developed in recent years to estimate body condition in polar bears (Atkinson and Ramsay 1995, Atkinson et al. 1996a) and brown bears (*Ursus arctos*; Hilderbrand et al. 2000), and is calculated as the proportion of fat to lean body mass (kg/kg). The values for fat and lean body mass are calculated based on the results obtained from body composition analysis by deuterium dilution (Farley and Robbins 1994). In this study, the paired Fatness Index (FI_{dd}) values for 18 bears were calculated from the results of 36 deuterium dilutions that were reported by Arnould (1990) and Atkinson et al. (1996a). The paired FI_{dd} values for the other two bears were determined first by applying the deuterium dilution technique to estimate fat and lean body mass as described by Farley and Robbins (1994), and then by calculating the FI_{dd} values as described by Atkinson and Ramsay (1995).

2.3 Results

The relationship between total body mass (TBM) and straight-line body length (SLBL) was described in both female and male polar bears by a curvilinear function of the form:

$$TBM = e^{\beta_0} \cdot SLBL^{\beta_1} \qquad \text{(Eqn. 2.2)}$$

Where e is the base of the natural logarithm, and β_0 and β_1 are the intercept and slope of the fitted line. Because the intercepts and slopes did not differ significantly ($Z_{\beta 0} = 1.65$ and $Z_{\beta 1} = 1.86$, $p \geq 0.07$) between the sex-specific equations, all data were pooled and a single regression equation was calculated (Fig. 2.1).

The distribution of body condition index (BCI) values for the 1072 bears conformed closely to the sigmoid curve defined by a normal frequency distribution (Fig. 2.2). No systematic trends were apparent in the scatter of data points that resulted from plotting the BCI values against SLBL (Fig. 2.3). Furthermore, the correlation between the two variables was not significant ($r = 0.005$, $p = 0.86$, $n = 1072$).

The variation in the mass of fat (range: 2.3 to 163.4 kg), skeletal muscle (range: 10.1 to 199.1 kg), potential energy tissue (fat + skeletal muscle; range: 12.4 to 362.5 kg), and structural tissue (skin and fur + bone + viscera; 18.4 to 155.7 kg) dissected from 31 polar bears was significantly associated with both the SLBL (range: 122 to 247 cm) and the BCI (range: -2.1 to +1.0) (Table 2.2). The SLBL was more closely correlated with the mass of most tissues, except for the dissectable fat for which the BCI was the stronger correlate. The proportion of the variation in tissue mass that was associated with the BCI was greater for the potential energy tissue than for the structural tissue as indicated by the respective increases in the coefficients of multiple determination (increase in R^2: 0.25 versus 0.01) following the inclusion of the BCI as a predictor variable in the regression model (Table 2.2; Fig. 2.4).

Four nomograms were constructed to allow estimates of the BCI to be made at the points of intersection between paired values for SLBL and TBM (Fig. 2.5a-d). The regression equations calculated for the different BCI lines were as follows:

$$TBM = e^{-10.3} \cdot SLBL^{3.09}, \text{ when BCI} = +2.0 \qquad \text{(Eqn. 2.3)}$$

$$TBM = e^{-10.5} \cdot SLBL^{3.10}, \text{ when BCI} = +1.5 \qquad \text{(Eqn. 2.4)}$$

$$TBM = e^{-10.7} \cdot SLBL^{3.11}, \text{ when BCI} = +1.0 \qquad \text{(Eqn. 2.5)}$$

$$TBM = e^{-10.6} \cdot SLBL^{3.07}, \text{ when BCI} = +0.5 \qquad \text{(Eqn. 2.6)}$$

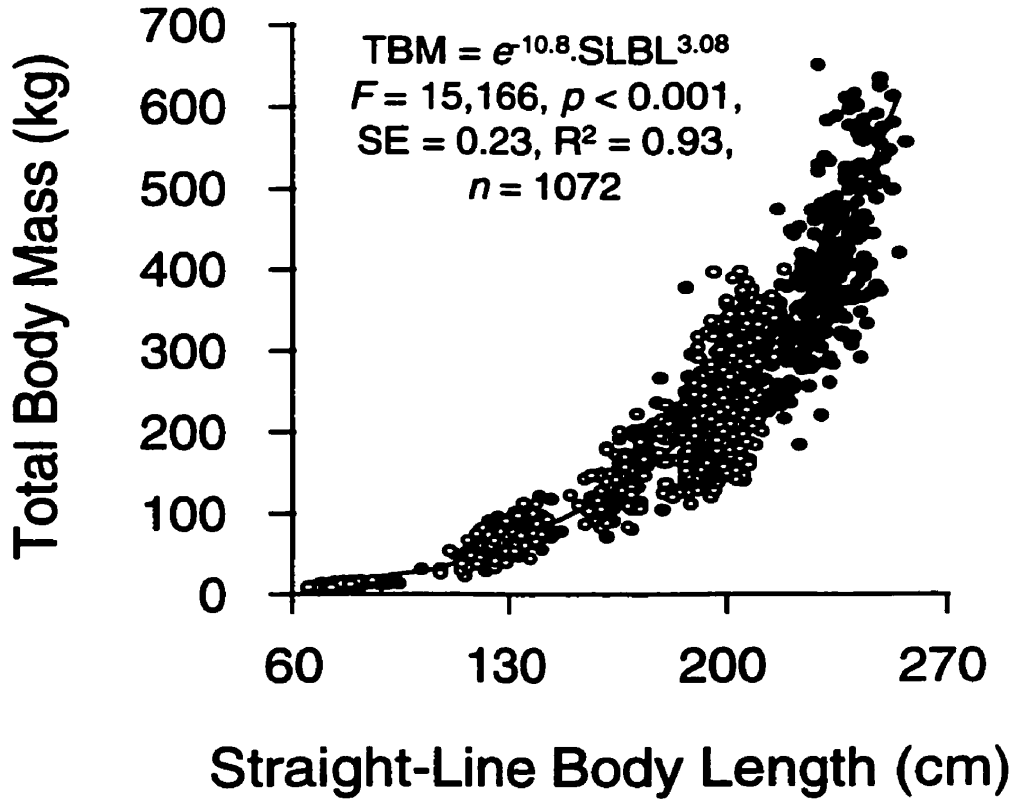


Figure 2.1 Curvilinear relationship between total body mass and straight-line body length in 1072 polar bears. Symbols represent females (○) and males (●).

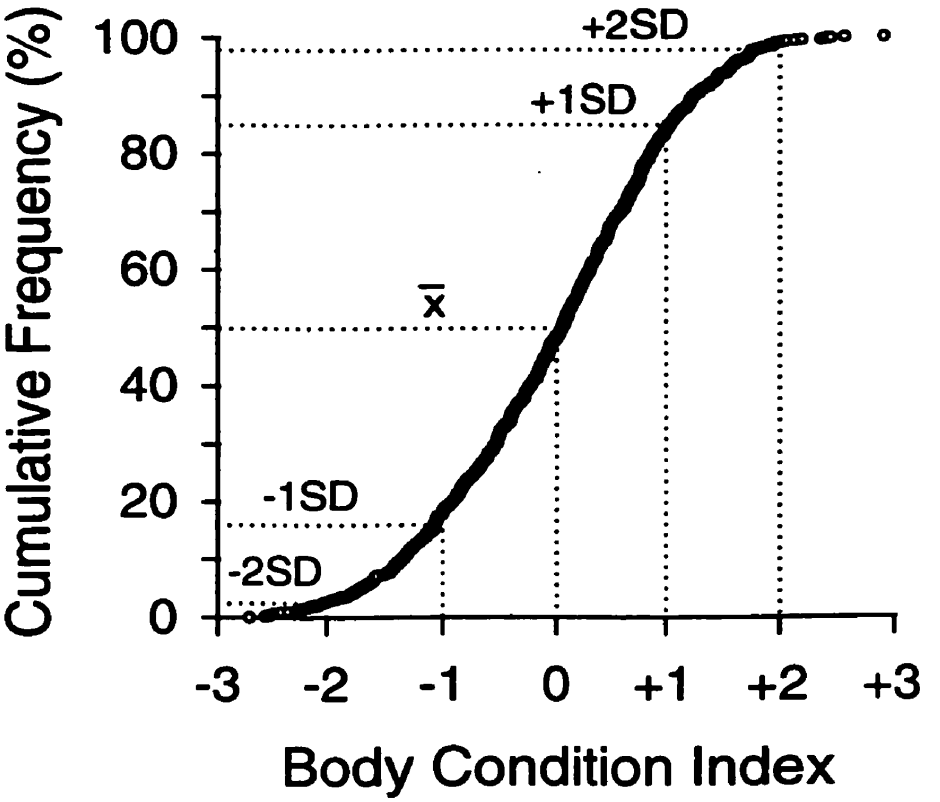


Figure 2.2 The cumulative frequency distribution of Body Condition Index (BCI) values for 1072 polar bears. The hatched lines show a close correspondence of the data distribution with a normal cumulative frequency distribution in that the cumulative frequency of BCI values at -2, -1, 0, +1, and +2 are approximately equal to the cumulative frequency of a normal distribution at -2 standard deviations (SD; 2.3%), -1SD (15.9%), the mean ($\bar{x}$; 50%), +1SD (65.9%), and +2SD (97.7%), respectively.

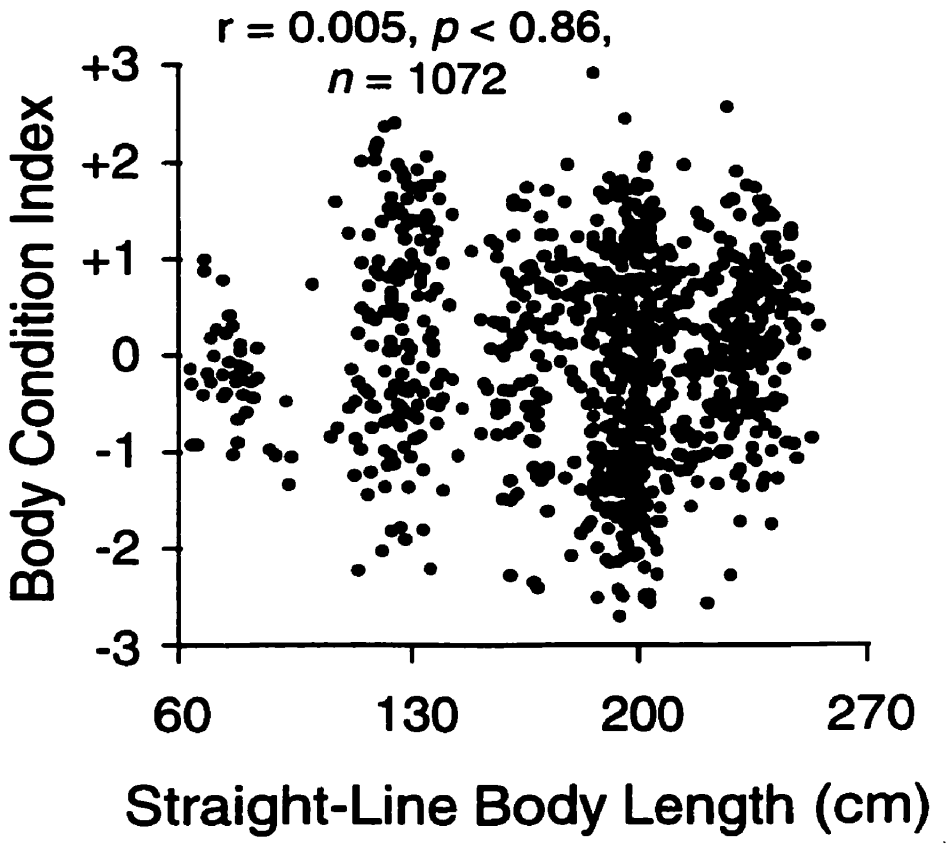


Figure 2.3 The association between the Body Condition Index (BCI) and straight-line body length (SLBL) in 1072 polar bears.

Table 2.2 The association between dissectible tissue mass (kg) and the predictor variables, straight-line body length (SLBL; cm) and the body condition index (BCI), in 31 polar bears.[a]

	Regression Models and Statistics					
	with highest correlated variable			with both predictor variables		
Tissue[b]	Model	R^2	SE	Model	R^2	SE
dissectible fat	$e^{(3.8 + 0.67BCI)}$	0.49	0.62	$e^{(0.70BCI - 12.1)} \cdot SLBL^{3.07}$	0.88	0.31
skeletal muscle	$e^{-13.5} \cdot SLBL^{3.38}$	0.87	0.22	$e^{(0.21BCI - 13.6)} \cdot SLBL^{3.43}$	0.97	0.11
potential energy tissue	$e^{-12.5} \cdot SLBL^{3.27}$	0.74	0.34	$e^{(0.35BCI - 12.6)} \cdot SLBL^{3.35}$	0.99	0.07
structural tissue	$e^{-10.6} \cdot SLBL^{2.83}$	0.97	0.09	$e^{(0.06BCI - 10.6)} \cdot SLBL^{2.84}$	0.98	0.07

[a] Models were determined by stepwise regression analysis with the dependent variable, tissue mass (kg), and the independent variables, SLBL (cm) and BCI. The association between the dependent and independent variable(s) were highly significant ($p < 0.001$) in all regression models. The coefficient of multiple determination (R^2) and the standard error of the predicted mean (SE) is presented for each model.

[b] Potential energy tissue mass is equal to the combined mass of dissectable fat and skeletal muscle. Structural tissue mass is equal to the combined mass of skin and fur, bone, and viscera.

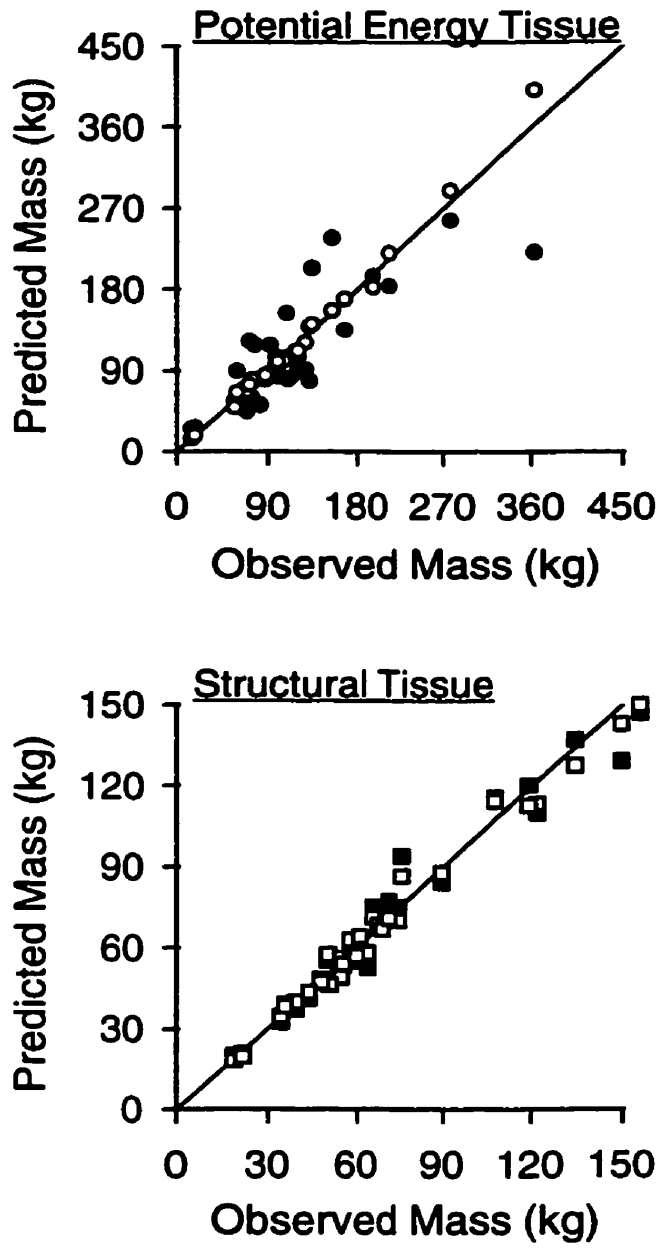


Figure 2.4 The association between the observed mass of dissectible tissue and that predicted by straight-line body length (SLBL) alone, or by the combination of SLBL and the Body Condition Index (BCI), in 31 polar bears. The regression models describing the different associations are presented in Table 2.2. The combination of SLBL and BCI (○) provide more accurate predictions of potential energy mass (dissectible fat mass + skeletal muscle mass) than does SLBL alone (●). However, the mass of structural tissue (skin and fur mass + bone mass + viscera mass) that is predicted by the combination of SLBL and BCI (□) is only slightly more accurate than that which is predicted by SLBL alone (■).

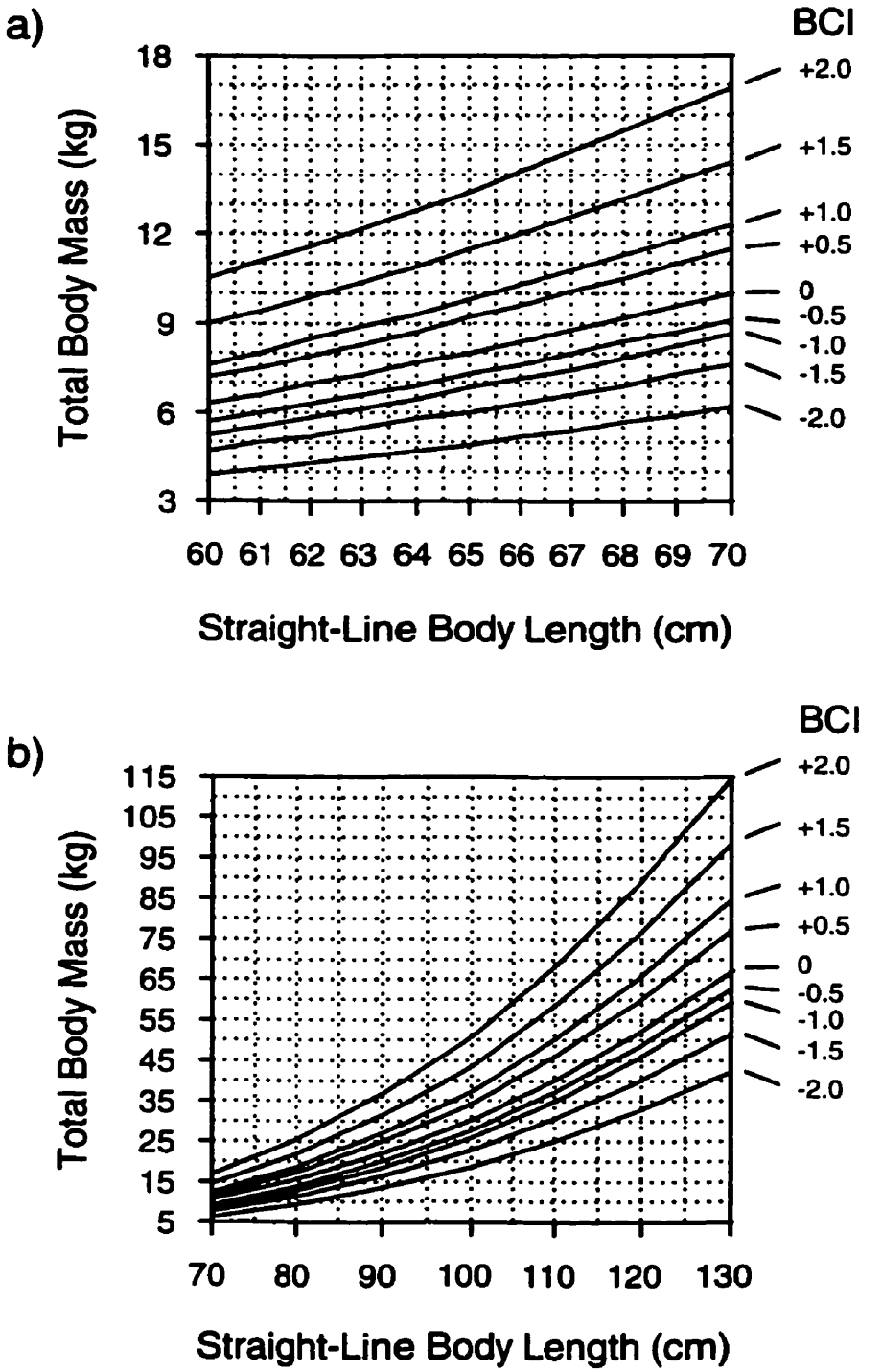


Figure 2.5 Nomograms for estimation of the Body Condition Index (BCI) over straight-line body length (SLBL) intervals of: a) 60 to 70 cm; b) 70 to 130 cm; c) 130 to 190 cm; and d) 190 to 270 cm. The estimated BCI value is found at the point of intersection for paired values of SLBL and total body mass.

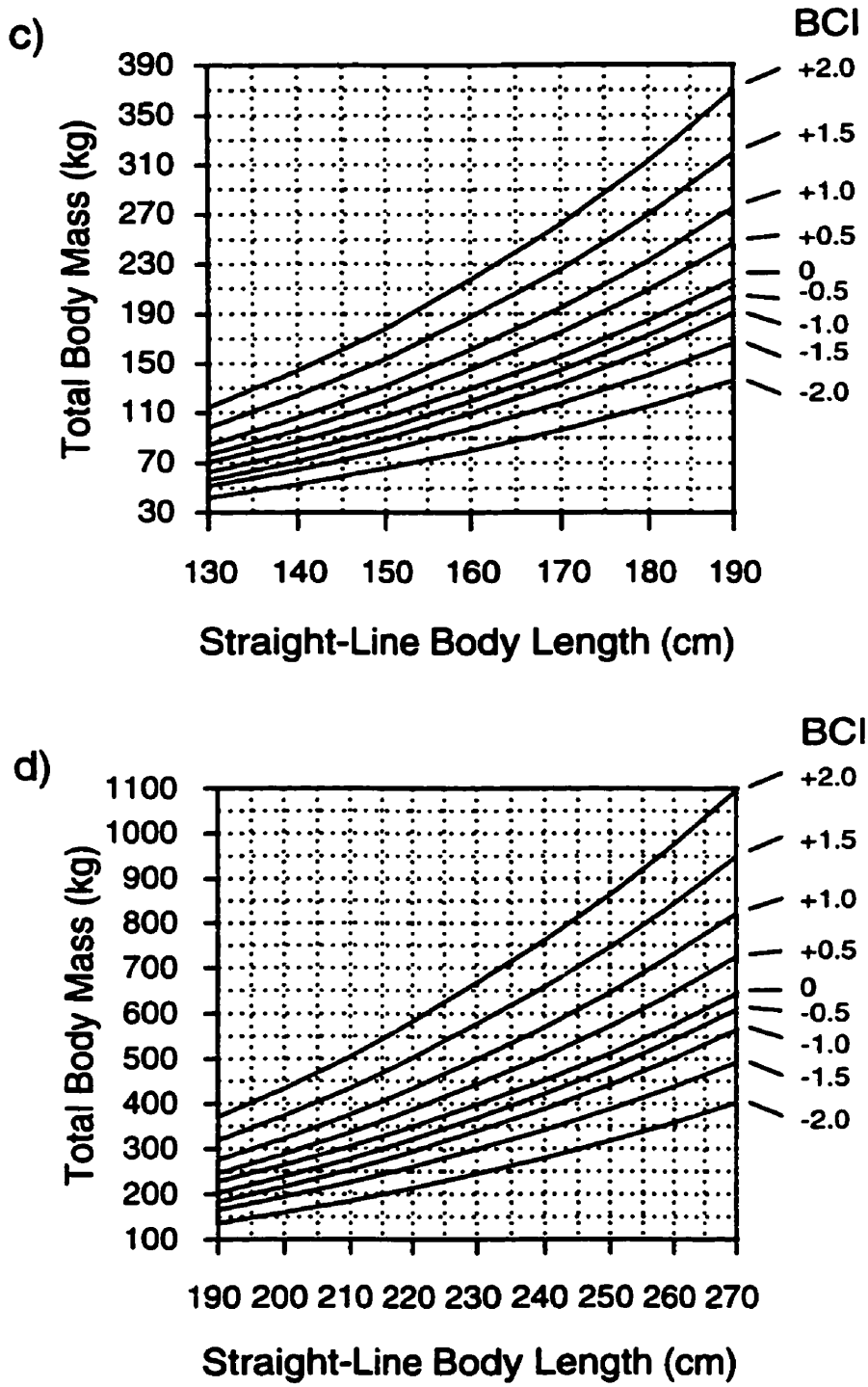


Figure 2.5 continued.

$TBM = e^{-10.8} \cdot SLBL^{3.08}$, when BCI = 0 (Eqn. 2.7)

$TBM = e^{-11.0} \cdot SLBL^{3.11}$, when BCI = -0.5 (Eqn. 2.8)

$TBM = e^{-11.0} \cdot SLBL^{3.10}$, when BCI = -1.0 (Eqn. 2.9)

$TBM = e^{-11.1} \cdot SLBL^{3.09}$, when BCI = -1.5 (Eqn. 2.10)

$TBM = e^{-11.3} \cdot SLBL^{3.09}$, when BCI = -2.0 (Eqn. 2.11)

The coefficients of multiple determination (R^2) ranged from 0.995 to 0.999 and the standard errors of the predicted means (SE) ranged from 0.027 to 0.035.

When comparing the BCI to the Quetelet Index (QI), the differences in mean values among months within the different adult classes were similar for both indices (Fig. 2.6). Within each adult class, mean BCI and QI values were greatest during July and August, intermediate during October, and least during April-May and November (one-way ANOVA: $F = 30.3$, $p < 0.001$; Tukey's Honestly Significant Difference test: $p < 0.05$). However, when comparing mean values among adult classes within some months, there were differences between the BCI and the QI. In July and in August, the mean BCI value for solitary females was significantly greater than both that for females accompanied by cubs and that for males. In contrast, there were no significant differences in mean QI value between solitary females and males at these times. Furthermore, in October, the mean BCI value for solitary females was greater (but not significantly different) than that for males, whereas the mean QI value for solitary females was less than that for males. The correlation between the QI and SLBL was positive and highly significant ($r = 0.66$, $p < 0.001$, $n = 1072$; Fig. 2.7).

When comparing the BCI to the Fatness Index (FI_{dd}), there were differences between indices both with respect to the ranking of condition values among the different bears at each of the two capture times, and with respect to the magnitude of change in the condition values for individual bears between the two capture times (Fig. 2.8). The capture interval varied from 20 to 24 days for nine fasting female polar bears that were accompanied by cubs, and from 66 to 92 days for 11 fasting male polar bears. There was no significant association between the BCI and FI_{dd} in their respective ranking of body condition values among the 20 bears at either capture time (Spearman correlation: r_s

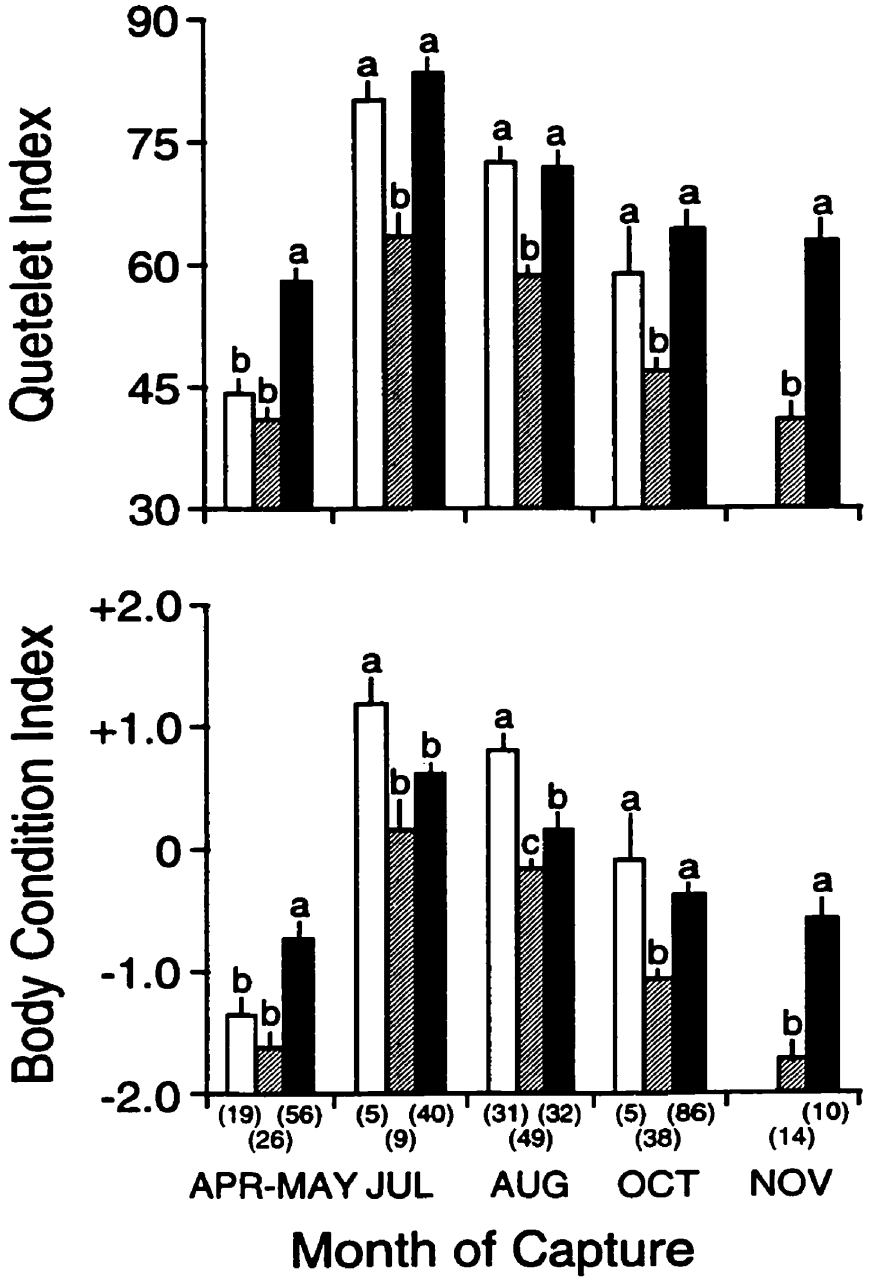


Figure 2.6 Estimation of the body condition of 420 adult polar bears, captured at different months of the year, by the Quetelet Index (QI) and the Body Condition Index (BCI). Within each month of capture, mean values + standard error are respectively represented by a vertical bar and line for solitary females (□), females accompanied by cubs (▨), and males (■). The number of bears of each adult class captured within each month is indicated in parentheses below the corresponding vertical bars. Different letters above the vertical bars within each month indicate significant differences between means as determined using Tukey's Honestly Significant Difference test.

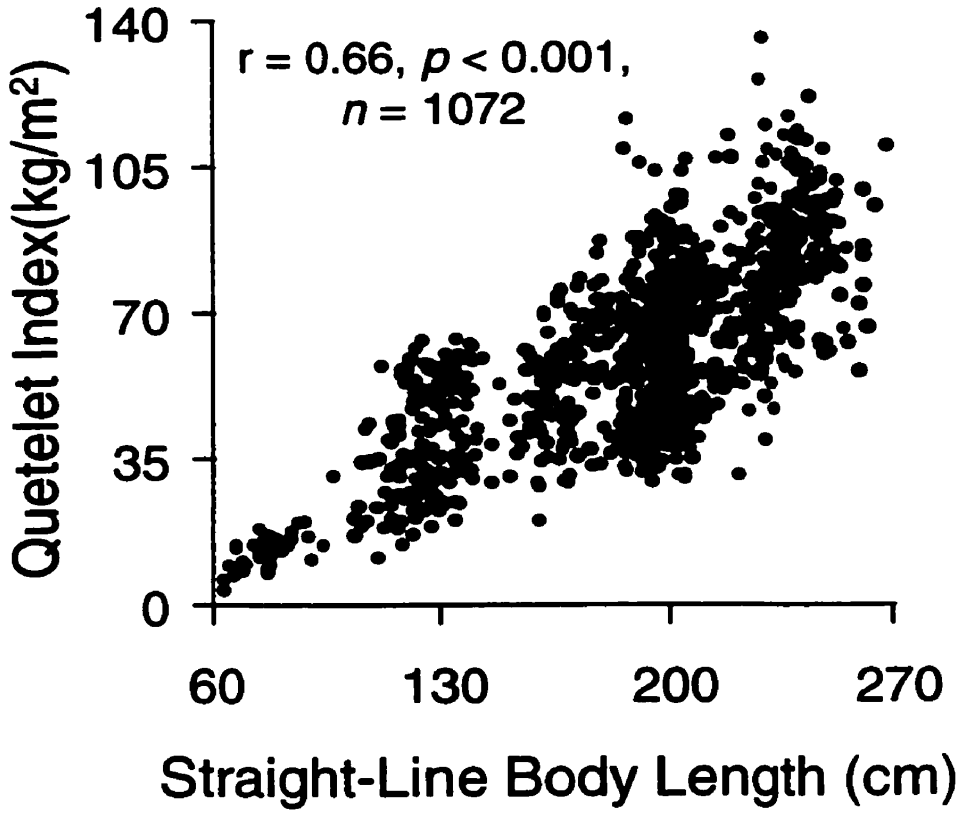


Figure 2.7 The association between the Quetelet Index (QI) and straight-line body length (SLBL) in 1072 polar bears.

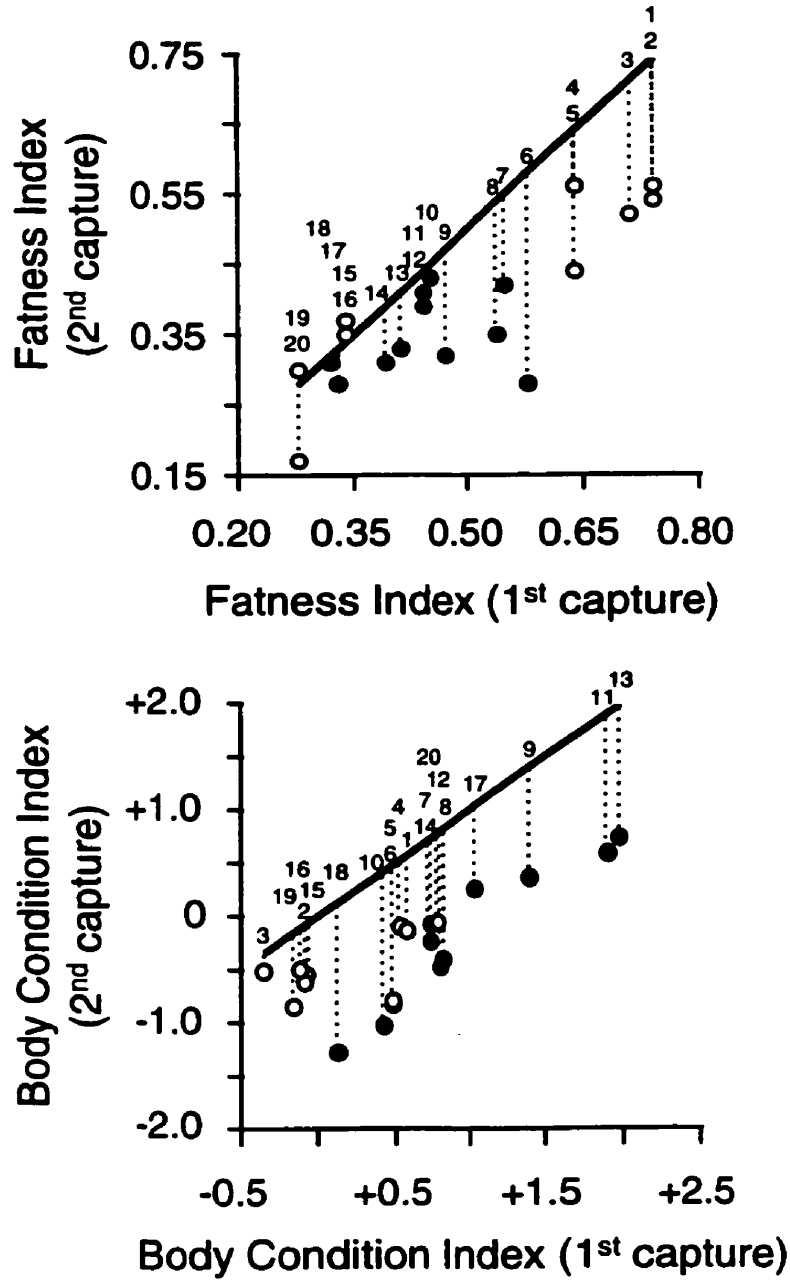


Figure 2.8 Estimation of the change in body condition of 20 polar bears, that were captured twice during the same year between July and October, by the Fatness Index (FI_{dd}) and by the Body Condition Index (BCI). The estimated body condition values of nine females (O) and 11 males (●) at first capture are plotted against the values at second capture. The hatched lines indicate the direction and magnitude of change in estimated body condition between captures. All points would align with the solid line if the estimate of body condition remained unchanged between captures. The numbers above the solid line correspond with individual data points below the line and provide a unique identification for each bear. The identification numbers indicate the ranking of the FI_{dd} values of bears at first capture relative to each other in descending order (i.e., the FI_{dd} value at first capture was highest for Bear 1 and lowest for Bear 20). Fatness Index and BCI values are calculated from data reported by Arnould (1990) and Atkinson (1996a).

= -0.13, $p = 0.58$ at first capture; $r_s = -0.07$, $p = 0.77$ at second capture). There also was no significant association between the BCI and FI_{dd} in their respective ranking of the change in body condition values among the 20 bears between the two capture times ($r_s = -0.01$, $p = 0.97$).

To further investigate the lack of association between the FI_{dd} and the BCI, Fatness Index values were calculated for 31 polar bears that were dissected by Cattet (1990), and for 11 polar bears for which whole body chemical composition was determined by Watts (1983; five bears) and Cattet (1988; six bears) (Fig. 2.9). The Fatness Indices based on tissue dissection ($FI_{dissect}$) and whole body chemical composition ($FI_{chemical}$) were calculated as:

$FI_{dissect}$ = dissectible fat mass in kg ÷ (TBM − dissectible fat mass in kg) (Eqn. 2.12)

$FI_{chemical}$ = whole body lipid content in kg ÷ (TBM − whole body lipid content in kg) (Eqn. 2.13)

The BCI was positively and significantly correlated with both the $FI_{dissect}$ (Pearson correlation: $r = 0.84$, $p < 0.001$, $n = 31$) and the $FI_{chemical}$ ($r = 0.76$, $p < 0.01$, $n = 11$), but was not correlated with the paired FI_{dd} values of 20 bears ($r = 0.14$, $p = 0.39$, $n = 40$).

2.4 Discussion

2.4.1 Development of the BCI

The primary objective of this investigation was to develop a body condition index (BCI) for polar bears which could be measured easily and used to compare among individual animals regardless of sex, age, reproductive class, geographical population, or season-of-capture.

The BCI is easy to measure. It is determined from two parameters, straight-line body length (SLBL; the straight-line distance from the tip of the nose to the end of the last tail vertebra) and total body mass (TBM), that are routinely measured during the handling of captured bears. Furthermore, the use of nomograms based on SLBL and TBM values provides rapid determination of BCI values without complex calculations (Fig. 2.5a-d). There is, however, potential for measurement error to have a small effect on the determination of BCI values because variation in the measurement of

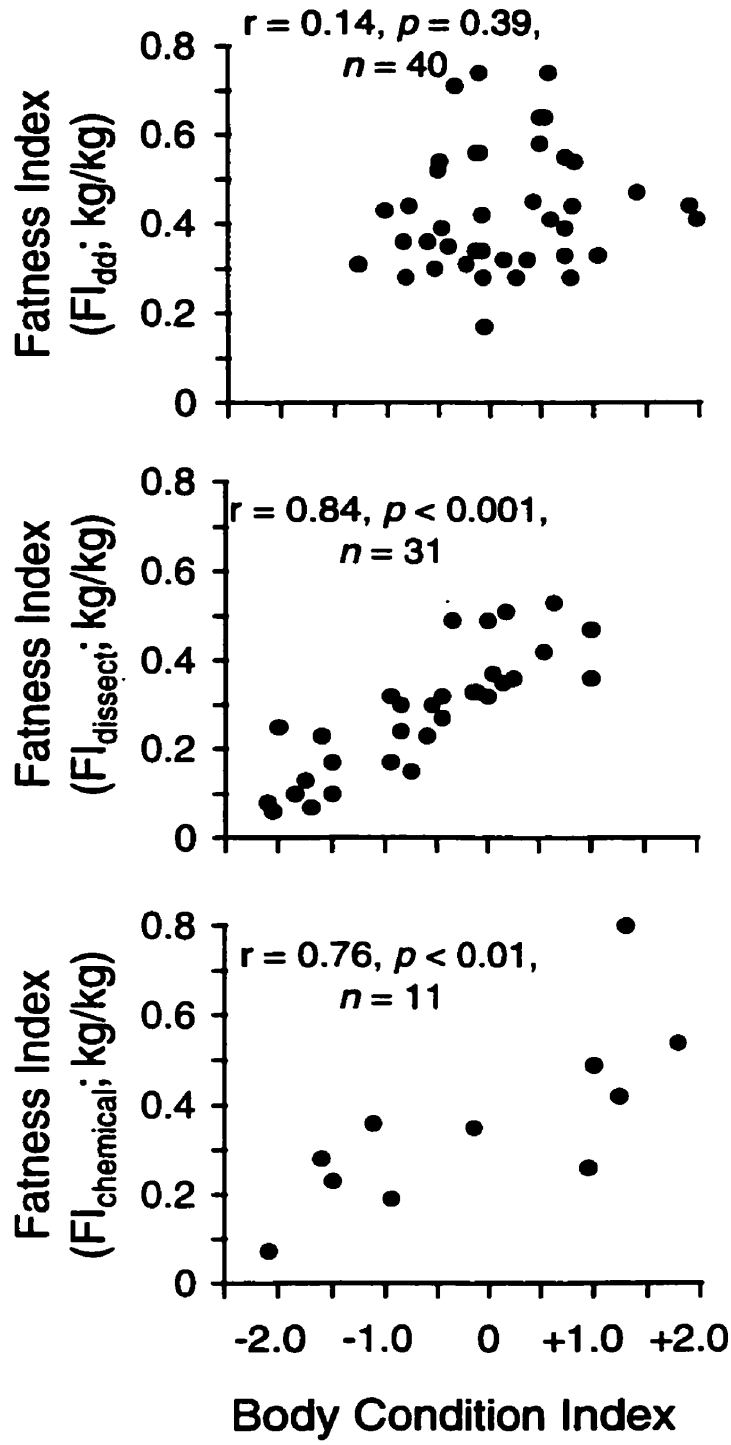


Figure 2.9 The association between the Fatness Index and the Body Condition Index (BCI) in polar bears. The Fatness Index is calculated based on the results of deuterium dilution (FI_{dd}; $n = 40$), the results of tissue dissection ($FI_{dissect}$; $n = 31$), and the results of whole body chemical composition ($FI_{chemical}$; $n = 11$). Fatness Index and BCI values are calculated from data reported by Watts (1983), Cattet (1988), Arnould (1990), Cattet (1990), and Atkinson (1996a).

SLBL can occur both among and within individual persons (Cattet 1990, Eason et al. 1996, Cattet et al. 1997). More significant error in BCI determination would be expected to occur if TBM is estimated by morphometric measurements (e.g., heart girth), instead of measured by weigh scale, because measurement error is compounded by the regression equation used to predict TBM (Cattet et al. 1997).

The BCI can be used to compare among individual animals regardless of sex, age, reproductive class, geographical population, or season-of-capture. This is because it was developed from a sample population that was large in number; representative of all sex, age, and reproductive classes; was comprised of members of three geographical populations; and also was comprised of polar bears captured during most months of the year (Table 2.1). Furthermore, the ranges of values for SLBL (62 to 256 cm) and TBM (5.9 to 654.0 kg) reported in this study are as large as the ranges of values that have been reported previously for polar bears in these three geographical populations (Kolenosky et al. 1989, Atkinson et al. 1996b, Derocher and Stirling 1998), and also are as wide as values reported for polar bears in other populations (Durner and Amstrup 1996, Derocher and Stirling 1998). Finally, because the BCI is the standardized residual for the curvilinear relationship between SLBL and TBM (Figs. 2.1 to 2.3), it can be compared among bears without the potentially confounding effect of variation in body size (Reist 1985, Piersma and Davidson 1991).

Although the BCI is a continuous variable that has no units and ranges in value from -3 to +3, it is sensitive to the abundance of potential energy stored in the body tissues of a polar bear relative to its body size. This was established by demonstrating that variation in the BCI values of 31 killed polar bears accounted for a large proportion of variation (25%) in the mass of potential energy tissue (dissectible fat + skeletal muscle) while accounting for only a small proportion (1%) of variation in the mass of structural tissue (skin and fur + bone + viscera) (Table 2.2 and Fig. 2.4).

2.4.2 Comparison of the BCI with Other Indices of Body Condition

To evaluate the use of the BCI, it was assessed with two other indices that have been used in recent years to measure body condition in free-ranging polar bears (Atkinson and Ramsay 1995, Atkinson et al. 1996a, Stirling et al. 1999). Assessment of the BCI with one of the indices, the Quetelet Index (QI), allowed comparison between these indices in a situation where the body condition of different bears captured at different times was being determined (i.e., cross-sectional data). Assessment of the BCI with the other index, the Fatness Index (FI_{dd}), allowed comparison between these indices in a situation where the change in body condition of the same bears captured at two different times was being determined (i.e., longitudinal data).

The pattern of differences in the mean body condition of the different classes of adult polar bears among months was similar between the BCI and the QI. The pattern of differences among adult classes within some months, however, were different between the two indices. The mean BCI values for solitary females during July and August were greater than those for males, and those for females accompanied by cubs, during the same months (Fig. 2.6). These results agree with observations that solitary adult females captured during summer often are pregnant and obese (Ramsay and Stirling 1988, Derocher et al. 1992) and that, relative to other adult classes, solitary females must be in good body condition to maintain their pregnancy and undergo lactation while in a fasting state (Watts and Hansen 1987, Atkinson and Ramsay 1995). In contrast, the mean QI values for solitary females were similar to those for males during July and August. This could be explained by the fact that the QI is affected by body size as was demonstrated by the strong positive correlation between SLBL and the QI (Fig. 2.7). As a result of this size-dependency, longer bears (e.g., adult males) were more likely to have higher QI values than were shorter bears (e.g., adult females) independent of any differences in body condition.

There was little, if any, correspondence between the BCI and the FI_{dd} both with respect to the ranking of condition values among different bears at each of two capture times, and with respect to the magnitude of change in the condition values for individual bears between the two capture times (Fig. 2.8). One potential explanation for a lack of agreement between these indices may be

that they were not developed to reflect the same definition for body condition. BCI values vary as the proportion of potential energy tissue mass (fat + skeletal muscle) varies in relation to the proportion of structural tissue mass. In contrast, FI_{dd} values reflect the proportion of fat mass in relation to the proportion of lean body mass, for which skeletal muscle is a component (Atkinson and Ramsay 1995, Atkinson et al. 1996a).

To determine if "different definitions of body condition" could explain a lack of agreement between these indices, BCI and Fatness Index values were calculated for 31 polar bears that were dissected by Cattet (1990). It was necessary, however, to calculate the Fatness Index values as the mass of dissectible fat divided by the mass of remaining body tissue (termed $FI_{dissect}$) because deuterium dilutions had not been done in these bears. Nevertheless, there was a significant and positive association between the BCI and the $FI_{dissect}$ values which did not support the proposed explanation (Fig. 2.9). BCI and Fatness Index values also were calculated for 11 bears for which whole body chemical composition had been determined by Watts (1983) and Cattet (1988). For these bears, Fatness Index values were calculated as the mass of chemically-extracted lipid divided by the TBM less the mass of chemically-extracted lipid (termed $FI_{chemical}$). Again, however, there was a significant and positive association between the BCI and $FI_{chemical}$ values (Fig. 2.9). Together, these results suggest the lack of agreement between the BCI and the FI_{dd} could not be explained by different definitions of body condition. These findings do, however, caution against the use of an isotope dilution model developed in black (*Ursus americanus*) and brown bears (Farley and Robbins 1994) to estimate body lipid content in polar bears, and instead point toward a need to determine the specific relationship between the results of deuterium dilution and whole body chemical composition for polar bears (see Appendix A).

3. ASPECTS OF PROTEIN AND AMINO ACID METABOLISM IN FREE-RANGING ADULT POLAR BEARS

3.1 Introduction

As time without food is extended, the distinction between fasting and starvation is determined primarily by the ability to spare protein from net catabolism. In fasting, protein loss is regulated in such a way as to maintain organ function and integrity. In starvation, protein loss exceeds a critical threshold beyond which organ function is compromised (Young and Scrimshaw 1971, Castellini and Rea 1992). The ability to spare protein is highly variable among species. The exceptional capacity of penguins, seals, and bears to survive prolonged fasts lasting weeks to months has been described previously (Castellini and Rea 1992). In these animals, protein loss is reduced either through a marked decrease in protein catabolism, as occurs in penguins and seals (Robin et al. 1988, Nordoy et al. 1990), or through nitrogen recycling, as occurs in black and brown bears (*U. americanus* and *U. arctos*) (Nelson et al. 1975, Barboza et al 1997).

Polar bears (*Ursus maritimus*) represent an extreme of fasting-adapted mammals (see Chapter 1), but little is known about their ability to spare body protein from net catabolism. Because changes that occur in the ratio of serum urea to serum creatinine at the commencement of fasting are similar among polar, black, and brown bears, it has been speculated that polar bears may use the same efficient protein-sparing mechanisms that are used by other bear species (Derocher et al. 1990, Ramsay et al. 1991). Estimation of the change in the body composition of fasting polar bears has determined, however, that the catabolism of protein appears to meet a significant proportion of energy demands in some individual bears (Atkinson et al. 1996a, but see Appendix A).

To further examine protein-sparing in polar bears, plasma concentrations of proteins, enzymes, free amino acids, and nitrogen end-products were measured in free-ranging polar bears

captured throughout the year to determine the association of these plasma constituents with nutritional state (feeding versus fasting), sex (female versus male), and body condition (lean versus fat). The activities and concentrations of enzymes and metabolites that are involved in the ornithine-urea cycle and intermediary metabolism also were measured in the livers of killed polar bears.

3.2 Materials and methods

3.2.1 Animals and samples

Thirty-nine, free-ranging, adult polar bears were captured from April to November, 1995. All bears were located by helicopter and chemically immobilized with Telazol® (8 to 10 mg/kg intramuscularly) using remote injection equipment (Stirling et al. 1989). Seventeen bears were captured during spring (April and May) while they were hunting seals on arctic sea-ice near Resolute Bay, Nunavut, Canada (74°00' to 76°00'N and 93°00' to 100°50'W). Twenty-two bears were captured during summer and fall (July to November) while they were fasting on land along the west coast of Hudson Bay near Churchill, Manitoba, Canada (57°00' to 58°50'N and 92°25' to 94°15'W).

Bears were weighed (± 0.5 kg) with an electronic load scale while suspended in a sling. Body length was measured by positioning bears in sternal recumbency and measuring the straight-line distance (± 0.5 cm) between the distal tips of nose and tail. Body condition of captured bears was quantified by a body condition index (BCI) based on the relationship between body mass and length (see Chapter 2). Ages of bears previously captured were obtained from a national database (Canadian Wildlife Service, Edmonton, Alberta). The ages of bears not previously captured were determined by extracting, sectioning, staining, and counting cementum annuli on one first premolar tooth (Stoneberg and Jonkel 1966). Bears 5 years of age or older were classified as adults (Lunn et al. 1997).

Samples of frozen liver tissue and plasma collected during 1993-94 from four adult male polar bears killed by Inuit hunters during spring, and from a single adult male polar bear killed by conservation enforcement officers during fall, were obtained to measure the activities and

concentrations of enzymes and metabolites involved in the ornithine-urea cycle and intermediary metabolism. All liver samples were collected and frozen in liquid nitrogen at the time of death.

3.2.2 Blood analysis

Twenty milliliters of blood was collected from the jugular vein into Vacutainer® tubes containing sodium heparin (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). Blood samples were cooled immediately following collection and were centrifuged within 12 hours to collect and freeze plasma. The packed cell volume (PCV) was determined using the microhematocrit technique (Bush 1991). Plasma total protein, albumin, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (γ-GT) were determined by the Clinical Pathology Laboratory at the Western College of Veterinary Medicine, University of Saskatchewan, using an Abbott Spectrum® Series II biochemistry analyzer (Abbott Laboratories Diagnostics Division, Abbott Park, Illinois 60064, USA). The concentration of the globulin fraction was calculated by subtracting the albumin concentration from the total protein concentration. Globulin fractions (α_1, α_2, β, and γ) were separated and quantified by protein electrophoresis using cellulose acetate as the supporting medium. Plasma free amino acids and ammonia were determined at the University of Illinois Veterinary Medical Clinic, Champaign-Urbana, using high performance liquid chromatography (Beckman System 6300 high performance amino acid analyzer; Beckman Coulter, Inc., Fullerton, California 92834-3100, USA).

3.2.3 Liver enzyme and metabolite analysis

Samples of frozen liver tissue from five polar bears were obtained to measure the activity of carbamoyl phosphate synthetase I (CPS-I), ornithine transcarbamoylase (OTC), argininosuccinate synthetase (AS), argininosuccinate lyase (AL), arginase, glutamine synthetase (GS), glutamate dehydrogenase (GDH), AST, ALT, lactate dehydrogenase (LDH), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), and 3-hydroxyacyl-coenzyme A dehydrogenase

(HOAD). Liver concentrations of urea, ammonia, glutamine, glutamate, and uric acid also were measured.

The activities of ornithine-urea cycle enzymes, GS, and GDH were determined at the Department of Zoology, University of Guelph, using a Varian DMS 100S spectrophotometer (Varian, Inc., Palo Alto, California 94304, USA) at 340 nm and at 10°C. Liver tissue extracts were prepared for determination of the activities of CPS-I, OTC, AS, AL, arginase, GS, and GDH by a modification of the procedure described by Korte et al. (1997). Frozen tissue (500 mg) was thawed, minced, homogenized with a Brinkmann Polytron™ homogenizer (Brinkmann Instruments, Inc., Westbury, New York 11590-0207, USA) in 1:9 w/v of extract buffer (50 mM 4-2-hydroxyethyl-1-piperazineethanesulfonic acid [HEPES] buffer, 50 mM KCl, 0.5 mM ethylenediamine tetra-acetic acid [EDTA], 1.0 mM dithiothreitol [DTT], 10 mM adenosine triphosphate [ATP], 25 mM $MgSO_4$, and 5.0 mM $NaHCO_3$), and sonicated (~30 sec). Homogenates were centrifuged at 14,400g for 10 minutes. Low molecular weight (< 5000 g/mole) substrates and effectors were removed from the supernatant using a 2.5 × 10 cm (~60 ml) Sephadex G-25 column. Assays were conducted according to the procedures described by Wright et al. (1992) and Wright et al. (1995), and the assay conditions are provided in Table 3.1.

Assays for all other enzymes were conducted at the Department of Animal Science, University of British Columbia. For these, liver tissue extracts were prepared by homogenizing thawed pieces of liver in a buffer (1:4 w/v) consisting of 50 mM imidazole and 1.0 mM EDTA (sodium salt), adjusted to pH 7.40 with HCl at room temperature. The homogenate was centrifuged for 1 minute at 12,000g and the supernatant was used in subsequent assays. Assays were conducted according to the procedures described by Vijayan et al. (1991), and the assay conditions are provided in Table 3.1.

Protein concentrations for ALT, AST, LDH, PEPCK, PK, and HOAD activities were determined using the bicinchoninic acid protein microplate assay described by Smith et al. (1985). The PK activity ratio was determined as the activity of PK at low PEP concentration as a proportion of the PK activity at high PEP concentration (Vijayan et al. 1997).

Table 3.1. Assay conditions used for the determination of liver enzyme activity in polar bears.

Enzyme	Conditions
CPS	• tissue extract was assayed both with ammonia + N-acetylglutamate and with ammonia alone, in addition to 100 mM ATP, 200 mM $MgSO_4$, 75 mM ornithine, 200 mM $NaHCO_3$, and 1 unit of OTC. The CPS-I activity was calculated by subtracting the CPS activity in the presence of ammonia alone from the activity with ammonia + N-acetylglutamate present.
OTC	• 75 mM ornithine, 50 mM carbamoyl phosphate, 50 mM KCl, 0.5 mM DTT, and 0.25 mM EDTA in 50 mM HEPES buffer.
AS	• 50 mM aspartate, 60 mM citrulline, 100 mM ATP, 200 mM $MgSO_4$, 100 U/ml phosphoenolpyruvate (PEP), 250 mM KH_2PO_4, 450 U/ml PK, 1000 U/ml arginase, and 10 U/ml AL in 50 mM HEPES buffer.
AL	• 60 mM argininosuccinate and 1000 U/ml arginase in 50 mM HEPES buffer.
arginase	• 100 mM arginine, 50 mM $MnCl_2$, and 100 mM glycine.
GDH	• 7.0 mM KCl, 28 mM $KHCO_3$, 5.0 mM KH_2PO_4, 50 mM $(NH_4)_2SO_4$, 1.0 mM EDTA, 1.0 mM ADP, 0.2 mM NADH, and 7.0 mM α-ketoglutarate (α-KG) in 30 mM HEPES buffer.
GS	• 40 mM imidazole-HCl buffer, 60 mM glutamine, 15 mM hydroxylamine-HCl, 3.0 mM $MnCl_2$, 20 mM sodium arsenate, and 0.4 mM ADP.
ALT	• 10 mM α-KG, 50 mM alanine, 25 μM pyridoxal 5'-phosphate, 0.2 mM NADH, and excess LDH.
AST	• 10 mM α-KG, 25 mM aspartate, 50 μM pyridoxal 5'-phosphate, 0.32 mM NADH, and excess glycerol-stabilized malate dehydrogenase (MDH).
LDH	• 1.0 mM pyruvate and 0.12 mM NADH.
PEPCK	• 9.0 mM PEP, 0.6 mM 2'-deoxyguanosine 5'-diphosphate, 20 mM $NaHCO_3$, 1.0 mM $MnCl_2$, 0.15 mM NADH, and excess MDH.
PK	• 0.6 mM PEP, 20 mM KCl, 10 mM $MgCl_2$, 6.0 mM ADP, 0.3 mM NADH, and excess PK-free LDH.
HOAD	• 0.1 mM acetoacetyl CoA and 0.15 mM NADH.

Liver metabolite concentrations were determined at the Department of Zoology, University of Guelph. For these, frozen tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. The powdered tissue was deproteinized with 1:1 v/w of chilled 2 N $HClO_4$ and centrifuged at 14,000g for 10 minutes. Afterward, the powdered tissue was neutralized with $KHCO_3$ and centrifuged again. Urea was measured using the method of Rahmatullah and Boyde (1980). Commercial diagnostic kits were used to measure concentrations of glutamine and glutamate (Sigma #GLN-1), ammonia (Sigma #171-A), and uric acid (Sigma #685-10; Sigma Chemical Company, St. Louis, Missouri 63178, USA). All metabolite measurements were carried out at 4°C.

3.2.4 Statistical analysis

Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, Illinois, USA). Two-way analysis of variance (ANOVA) was used to determine differences in plasma constituents among nutritional states (feeding and fasting), and between sexes (Zar 1996). The body condition index (BCI) and drug dosage (mg/kg) were included as covariates. Student's *t*-test for two independent samples was used to compare globulin protein concentrations and amino acid groups between seasons, and the Mann-Whitney *U*-test was used to compare globulin protein concentrations between sexes within each season. Pearson (r) and first-order partial correlation ($r_{partial}$) analyses were used to identify associations among plasma constituents. Spearman correlation analysis (r_s) was used to determine the pairwise associations between values for plasma and liver constituents from killed polar bears (Zar 1996). For all statistical tests, significance was assigned when the probability of a Type I error was equal to or less than 0.05. All results are reported as mean ± standard error (SE).

3.3 Results

Packed cell volume (PCV) and the plasma concentrations of proteins, enzymes, nitrogen end-products, and most amino acid groups differed significantly by nutritional state, or sex, or a combination of these factors (Table 3.2). Fasting polar bears had higher concentrations of globulin

Table 3.2 Packed cell volume and concentrations of proteins, enzymes, amino acid (a.a.) groups, and nitrogen end-products in the plasma of feeding (captured during spring) and fasting (captured during summer-fall) adult polar bears.[a]

Plasma constituent	Feeding (spring) female (n = 9)	Feeding (spring) male (n = 8)	Fasting (summer-fall) female (n = 11)	Fasting (summer-fall) male (n = 11)	Factor or Covariate NS	sex	NS × sex	BCI
packed cell volume (%)	40.2±1.19 (32.5-43.5)	46.8±1.02 (44.0-51.0)	47.5±1.44 (39.0-53.5)	47.0±1.19 (42.0-56.0)	ns	ns	*	***
total protein (g/L)	69±0.9 (64-71)	73±0.9 (68-76)	75±1.0 (49-84)	81±1.9 (73-94)	ns	*	ns	**
albumin (g/L)	40±0.4 (39-43)	41±0.9 (38-44)	40±0.7 (29-46)	40±1.2 (36-46)	ns	ns	ns	ns
globulin (g/L)	28±0.6 (25-31)	31±0.6 (29-33)	35±0.9 (20-42)	41±1.9 (33-57)	**	**	ns	**
ALT (U/L)	12±1.7 (8-24)	22±1.8 (13-29)	8±0.7 (6-12)	8±1.0 (6-17)	***	**	**	**
AST (U/L)	36±2.6 (26-50)	59±7.3 (35-90)	28±2.1 (16-41)	26±1.2 (21-33)	***	*	**	**
γ-GT (U/L)	39± 6.9 (21-79)	109±16.6 (63-176)	55±2.9 (36-65)	41±8.0 (16-100)	**	*	**	ns
total a.a. (mmol/L)	4.4±0.44 (3.0-6.3)	4.1±0.12 (3.7-4.7)	3.7±0.17 (2.6-4.7)	4.4±0.15 (3.4-5.0)	ns	ns	*	ns
total a.a. nitrogen (mmol/L)	6.4±0.57 (4.4-8.7)	5.7±0.16 (5.1-6.5)	5.6±0.27 (3.8-8.2)	6.6±0.26 (4.9-9.4)	ns	ns	*	ns
essential a.a. (mmol/L)	1.3±0.19 (0.8-2.6)	1.4±0.09 (1.2-1.6)	1.2±0.07 (0.7-1.6)	1.5±0.05 (1.2-1.8)	ns	ns	ns	ns
non-essential a.a. (mmol/L)	3.1±0.31 (1.9-4.7)	2.7±0.15 (2.4-3.3)	2.5±0.11 (1.9-3.1)	3.0±0.11 (2.2-3.5)	ns	ns	*	ns
ammonia (μmol/L)	448±18.2 (370-536)	521±26.5 (526-582)	447±14.9 (389-543)	438±12.6 (390-501)	*	ns	*	ns
urea (mmol/L)	7.0±1.54 (1.7-15.8)	11.4±2.86 (3.0-23.1)	2.7±0.23 (1.7-4.4)	2.9±0.23 (1.4-3.8)	*	ns	ns	**
creatinine (μmol/L)	115±18.9 (50-235)	124±10.1 (92-176)	156±14.3 (88-226)	171±8.6 (138-235)	*	ns	ns	*
U/C ratio (mg/dl:mg/dl)	16.8±3.44 (2.7-32.4)	26.3±7.81 (5.5-53.1)	4.6±0.48 (2.5-7.6)	4.3±0.40 (2.2-6.8)	*	ns	ns	***

[a] Values reported as mean ± SE with range presented in parentheses. Statistical comparison made by two-way ANOVA with nutritional state (NS) and sex as the factors, NS × sex as the interaction, and the Body Condition Index (BCI) as the covariate. Significance indicated by "ns" for non-significance, * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

and creatinine; lower concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea; and a lower urea to creatinine (U/C) ratio, than did feeding bears. Male polar bears had higher concentrations of total and globulin protein in plasma than did females, irrespective of nutritional state. Feeding male bears also had higher concentrations of ammonia in plasma than did feeding females. Fasting female bears had a higher concentration of γ-glutamyltransferase (γ-GT) and lower concentrations of total amino acid, total amino acid nitrogen, and nutritionally non-essential amino acid in plasma than did feeding females. In male polar bears, the differences between feeding and fasting for these same constituents (i.e., amino acid groups and γ-GT) were opposite to that which occurred in females. The body condition index (BCI) covaried significantly with all constituents that differed significantly by nutritional state. The BCI also covaried significantly with the PCV and total protein concentration. The correlation between plasma total protein and the BCI was positive and remained highly significant when controlled for variation in PCV ($r_{partial} = 0.54$, $p < 0.001$). The BCI also correlated significantly with the plasma concentrations of globulin ($r = 0.47$, $p < 0.01$), ALT ($r = -0.38$, $p < 0.05$), AST ($r = -0.34$, $p < 0.05$), urea ($r = -0.50$, $p < 0.001$), and the U/C ratio ($r = -0.52$, $p < 0.001$). In all bears, the concentrations of albumin and nutritionally essential amino acid in plasma were similar between feeding and fasting. Neither the concentrations of proteins, enzymes, amino acid groups, and nitrogen end-products in plasma, nor the U/C ratio, correlated or covaried significantly with the dosage of anesthetic drug administered.

Fasting polar bears had significantly greater plasma concentrations of β- and γ-globulin proteins than did feeding bears (Fig. 3.1). The peaks of these fractions, however, were broad-based in the plasma samples of both feeding and fasting bears. Male polar bears had significantly greater plasma concentrations of globulin than did females irrespective of nutritional state, but there were no significant differences between sexes when comparing globulin fractions as a percentage of total globulin (Mann-Whitney *U*-test, $p > 0.05$ for each fraction).

The plasma concentrations of most free amino acids differed significantly by nutritional state, or sex, or a combination of these factors (Table 3.3). Fasting polar bears had higher concentrations of lysine, alanine, 3-methylhistidine, and taurine; and lower concentrations of

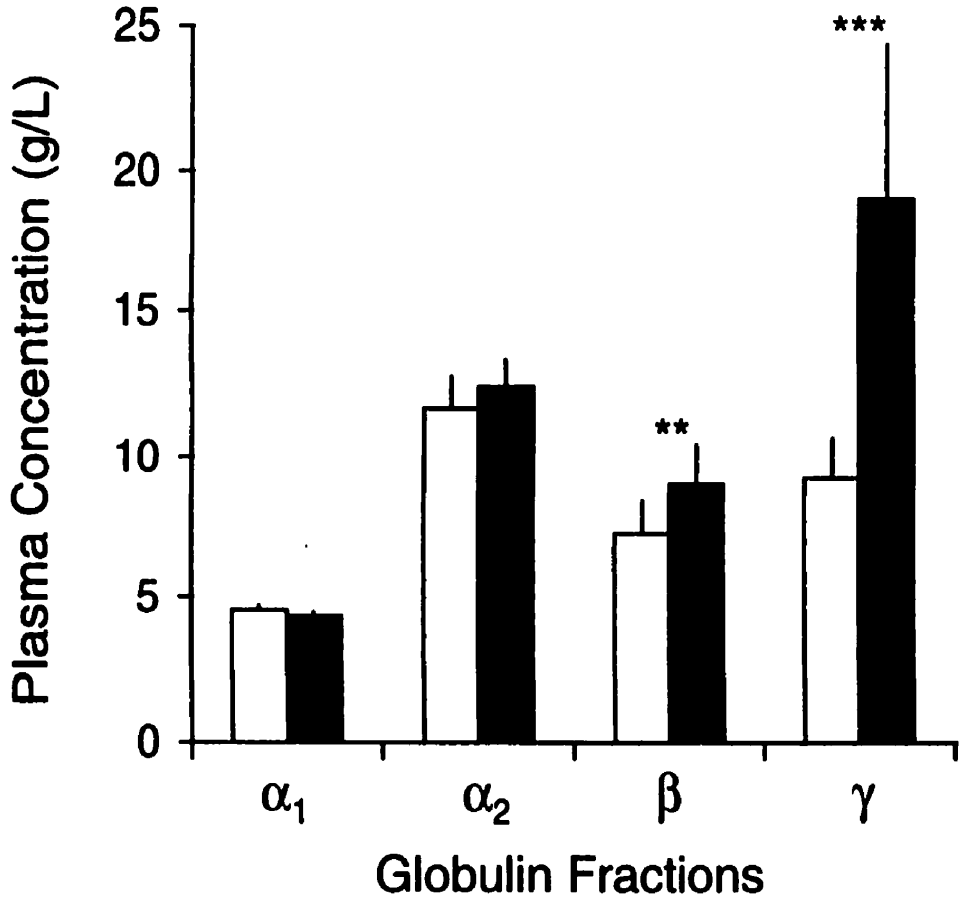


Figure 3.1 Plasma concentration of globulin protein fractions in feeding and fasting polar bears. The bars represent the mean values for feeding ($n = 9$; □) and fasting bears ($n = 9$; ■) with the standard deviation indicated by a vertical line. Mean values for each fraction were compared between feeding and fasting bears using a *t*-test for two independent samples, and significant differences are indicated by ** for $p \leq 0.01$ and *** for $p \leq 0.001$.

Table 3.3 Concentrations of free amino acids in the plasma of feeding (captured during spring) and fasting (captured during summer-fall) adult polar bears.[a]

Amino acid[b]	Feeding (spring) female ($n = 9$)	Feeding (spring) male ($n = 8$)	Fasting (summer-fall) female ($n = 11$)	Fasting (summer-fall) male ($n = 11$)	Factor or Covariate: NS	sex	NS × sex	BCI
1. Nutritionally essential amino acids								
arginine	135±9.8	120±7.5	150±9.2	162±8.5	ns	ns	*	**
histidine	96±8.6	89±7.4	90±3.2	94±3.3	ns	ns	ns	ns
isoleucine	70±9.2	101±12.6	77±8.7	91±5.9	ns	*	ns	ns
leucine	176±36.7	186±28.9	130±11.7	170±11.0	ns	ns	ns	ns
lysine	157±21.2	135±13.0	232±21.6	294±18.8	**	ns	*	***
methionine	18±1.8	24±1.9	21±1.9	25±2.1	ns	*	ns	ns
phenylalanine	68±6.6	75±4.3	74±4.5	81±3.7	ns	ns	ns	*
threonine	191±16.0	218±16.8	131±8.5	169±5.9	**	**	ns	**
tryptophan	106±15.3	134±10.8	99±6.5	111±11.9	ns	ns	ns	ns
valine	268±52.3	326±34.9	170±12.5	227±8.8	ns	ns	ns	*
2. Nutritionally non-essential amino acids								
alanine	467±63.7	461±38.0	581±45.1	645±40.0	*	ns	ns	*
α-aminobutyrate	34±3.6	37±2.8	34±3.3	32±1.8	ns	ns	ns	ns
asparagine	95±16.6	38±14.7	8±7.9	26±11.1	*	ns	**	**
aspartate	12±1.1	9±0.4	10±0.4	11±0.6	ns	ns	ns	*
carnosine	16±6.3	15±7.2	29±4.5	28±4.3	ns	ns	ns	*
citrulline	46±7.9	44±6.2	41±3.1	45±6.2	ns	ns	ns	ns
cystathionine	5±0.5	3±0.7	4±0.2	6±0.3	*	ns	**	ns
glutamate	263±31.3	464±48.8	367±28.4	302±21.6	ns	ns	**	ns
glutamine	755±44.8	599±54.8	587±36.7	802±54.2	ns	ns	**	ns
glycine	572±87.4	315±26.8	337±17.0	420±31.7	*	ns	**	ns
hydroxyproline	150±39.0	106±22.8	41±4.8	49±2.5	*	ns	ns	**
1-methylhistidine	2±1.1	4±3.8	5±1.6	4±1.2	ns	ns	ns	ns
3-methylhistidine	16±3.3	2±1.6	31±2.5	27±1.5	***	**	ns	***
ornithine	57±10.9	68±8.0	44±3.4	61±6.4	ns	ns	ns	ns
phosphoserine	9±0.4	9±0.8	7±0.3	6±0.2	*	ns	ns	***
proline	275±64.5	212±28.4	159±10.9	185±12.5	ns	ns	ns	*
serine	185±20.7	138±8.8	145±7.3	196±9.0	**	ns	**	ns
taurine	82±8.4	89±13.6	96±9.5	100±7.5	*	ns	ns	ns
tyrosine	66±7.9	74±3.7	41±3.2	51±4.1	***	*	ns	*

[a] Values reported as mean ± SE. Statistical comparison made by two-way ANOVA with nutritional state (NS) and sex as the factors, NS × sex as the interaction, and the Body Condition Index (BCI) as the covariate. Significance indicated by "ns" for non-significance, * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

[b] All concentrations are expressed as μmol/L.

phosphoserine, threonine, hydroxyproline, and tyrosine, than did feeding bears. Male bears had lower concentrations of 3-methylhistidine and higher concentrations of isoleucine, methionine, threonine, and tyrosine than did females, irrespective of nutritional state. Fasting female bears had a higher concentration of glutamate and lower concentrations of asparagine, cystathionine, glutamine, glycine, and serine in plasma than did feeding females. In male polar bears, the differences between feeding and fasting for these same amino acids were opposite to that which occurred in females. Fasting female bears had a significantly greater proportion of their total amino acid nitrogen occurring in amino acids that contained three (histidine, citrulline, 1-methylhistidine, and 3-methylhistidine) and four nitrogens (arginine and carnosine) than did feeding females (Fig. 3.2). Fasting male bears had a significantly lesser proportion of their total amino acid nitrogen occurring in amino acids that contained one nitrogen, and a significantly greater proportion of their total amino acid nitrogen occurring in amino acids that contained two (lysine, tryptophan, asparagine, cystathionine, glutamine, and ornithine) and four nitrogens, than did feeding males (Fig. 3.2). The BCI covaried significantly with all the amino acids that differed significantly by nutritional state, except taurine. The BCI also covaried significantly with the plasma concentrations of arginine, phenylalanine, valine, carnosine, and proline. Of the amino acids that covaried with the BCI, significant pairwise correlations occurred between the BCI and plasma concentrations of arginine ($r = 0.51$, $p < 0.001$), lysine ($r = 0.61$, $p < 0.001$), phenylalanine ($r = 0.33$, $p < 0.05$), threonine ($r = -0.43$, $p < 0.01$), valine ($r = -0.32$, $p < 0.05$), asparagine ($r = -0.36$, $p < 0.05$), hydroxyproline ($r = -0.39$, $p < 0.05$), 3-methylhistidine ($r = 0.53$, $p < 0.001$), and phosphoserine ($r = -0.53$, $p < 0.001$). There were no signficant pairwise correlations between the individual free amino acids and the dosage of anesthetic drug administered, or between the individual free amino acids and the PCV.

Many of the values for the plasma and liver constituents of a single fasting male polar bear killed during fall fell outside of the ranges of values of four feeding male polar bears killed during spring (Table 3.4). The plasma concentrations of total protein, globulin, and creatinine; the liver activities of argininosuccinate lyase (AL) and arginase; the pyruvate kinase (PK) activity ratio; and the liver concentrations of glutamine and uric acid were all greater in the fasting bear. The plasma

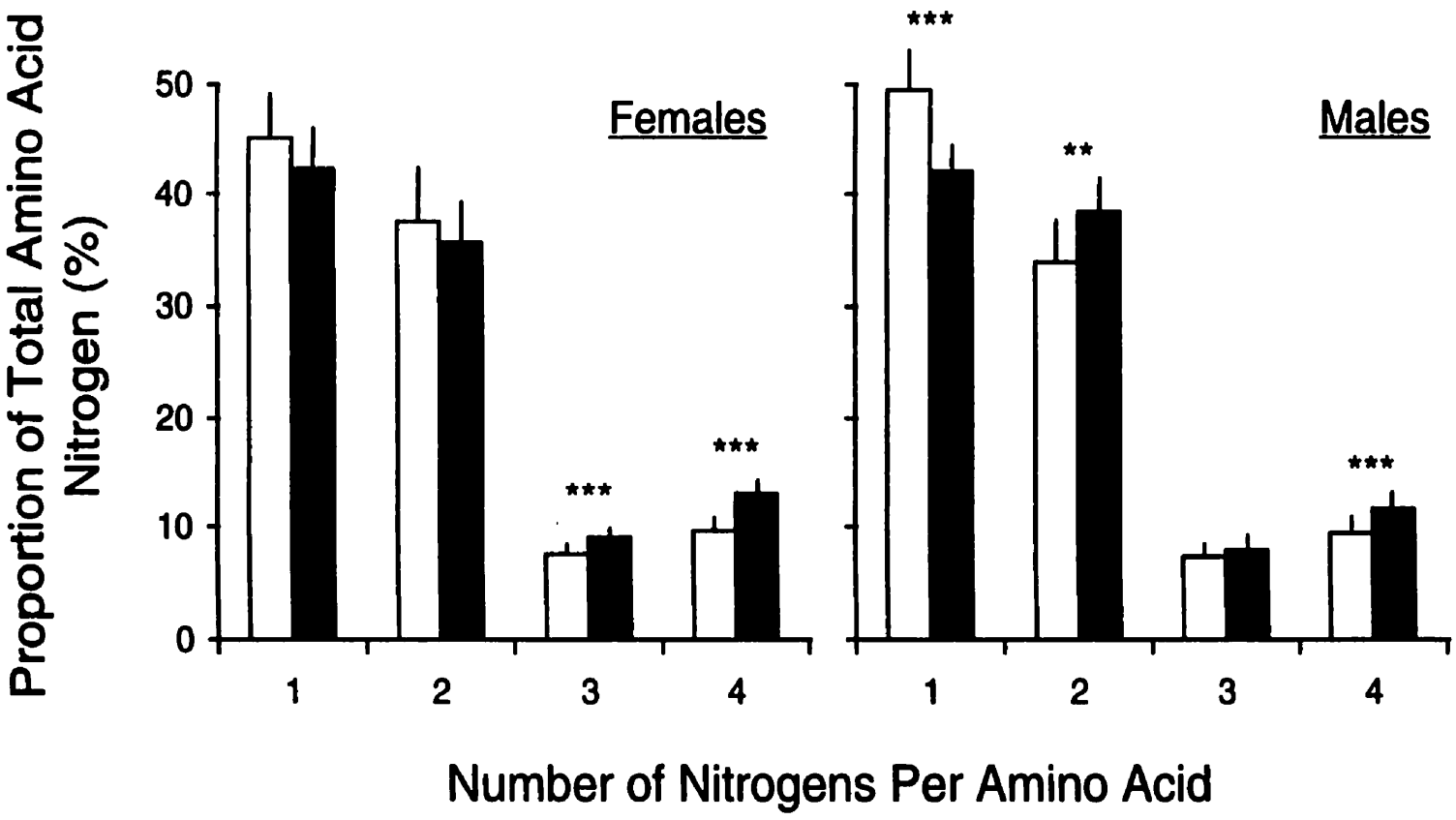


Figure 3.2 The distribution of nitrogen, as a proportion of total amino acid nitrogen, among plasma amino acids containing one, two, three, and four nitrogens in feeding and fasting polar bears. The bars represent the mean values for feeding (□) and fasting bears (■) with the standard deviation indicated by a vertical line. The proportion of total amino acid nitrogen within each class of amino acids was compared between feeding (nine females and eight males) and fasting bears (11 females and 11 males) using a *t*-test for two independent samples, and significant differences are indicated by ** for $p \leq 0.01$ and *** for $p \leq 0.001$.

Table 3.4 Body condition and the concentrations and activities of various metabolites and enzymes measured in the plasma and liver of four feeding (captured during spring) adult male polar bears, and one fasting (captured during fall) adult male polar bear.[a]

Variable or constituent	Feeding (spring)	Fasting (fall)	Fasting Value Relative to Feeding Values
1. Body condition			
BCI	−1.55 - −0.17	−0.40	0
2. Plasma metabolite or enzyme concentration			
total protein (g/L)	71 - 77	79	↑
albumin (g/L)	40 - 44	41	0
globulin (g/L)	29 - 33	38	↑
ALT (U/L)	13 - 29	7	↓
AST (U/L)	35 - 90	25	↓
γ-GT (U/L)	128 - 154	39	↓
urea (mmol/L)	2.7 - 9.8	0.8	↓
creatinine (μmol/L)	96 - 149	164	↑
U/C ratio	6.9 - 16.9	1.2	↓
3. Liver enzyme activity			
CPS-I (μmol/min/g protein)	4.1 - 8.2	3.9	↓
OTC (μmol/min/g protein)	563 - 800	497	↓
AS (μmol/min/g protein)	2.0 - 3.7	2.9	0
AL (μmol/min/g protein)	8.5 -12.3	15.9	↑
arginase (μmol/min/g protein)	437 - 841	911	↑
GDH (μmol/min/g protein)	101 - 163	151	0
GS (μmol/min/g protein)	22 - 79	54	0
ALT (μmol/min/g protein)	396 - 925	276	↓
AST (mmol/min/g protein)	6.1 - 11.6	4.8	↓
LDH (mmol/min/g protein)	8.1 -22.2	11.5	0
PEPCK (μmol/min/g protein)	57 - 130	111	0
PK (μmol/min/g protein)	466 - 1258	458	↓
PK activity ratio	0.24 - 0.31	0.40	↑
HOAD (mmol/min/g protein)	1.5 - 3.1	1.3	↓
4. Liver metabolite concentration (corrected for liver water content)			
glutamate (mmol/L)	51 - 63	24	↓
glutamine (mmol/L)	3.7 - 9.8	9.9	↑
ammonia (mmol/L)	2.1 - 18.0	1.8	↓
urea (mmol/L)	3.2 - 22.4	1.1	↓
uric acid (mmol/L)	24 - 234	278	↑

[a] Values are reported as the range from smallest to greatest for feeding bears. The fasting value relative to feeding values is indicated by 0 when the fasting value falls within the range of feeding values, or by ↑ when the fasting value falls above the range of feeding values, or by ↓ when the fasting value falls below the range of feeding values.

concentrations of ALT, AST, γ-GT, and urea; the plasma U/C ratio; the liver activities of carbamoyl phosphate synthetase I (CPS-I), ornithine transcarbamoylase (OTC), ALT, AST, PK, and 3-hydroxyacyl-coenzyme A dehydrogenase (HOAD); and the liver concentrations of glutamate, ammonia, and urea and were all greater in the feeding bears. In all five bears, the liver urea concentration correlated positively with CPS-I activity and the concentrations of plasma urea and liver ammonia, and correlated negatively with liver glutamine concentration (Fig. 3.3). The concentrations of ALT and AST in plasma and in liver were also significantly correlated (r_s =0.90, p < 0.05 for both enzymes).

3.4 Discussion

3.4.1 Season of capture, nutritional state, and the U/C ratio

The differences in the urea to creatinine (U/C) ratios between bears captured during spring and bears captured during summer and fall in this study (Table 3.2) supported the assumption that bears captured during spring and bears captured during summer-fall were respectively analogous to feeding bears and fasting bears. The high U/C ratios measured in many of the bears captured during spring were similar to values reported in previous studies (Derocher et al. 1990, Ramsay et al. 1991) and suggested that these bears were in a metabolic state consistent with feeding. Conversely, the low U/C ratios measured in many bears captured during summer and fall indicated that these bears were in a metabolic state consistent with fasting.

3.4.2 Association with nutritional state

The physiological role of an amino acid is difficult to ascertain from its plasma concentration alone because amino acids are in a state of constant and rapid flux between plasma and intracellular compartments (Adibi 1971, Abumrad and Miller 1983). The same is also true for many of the other plasma constituents measured in this study. Thus, additional information provided by the measurement of enzymes and metabolites in the plasma and liver of five killed polar bears (Table 3.4) was used to determine how anabolic and catabolic processes might differentially influence the

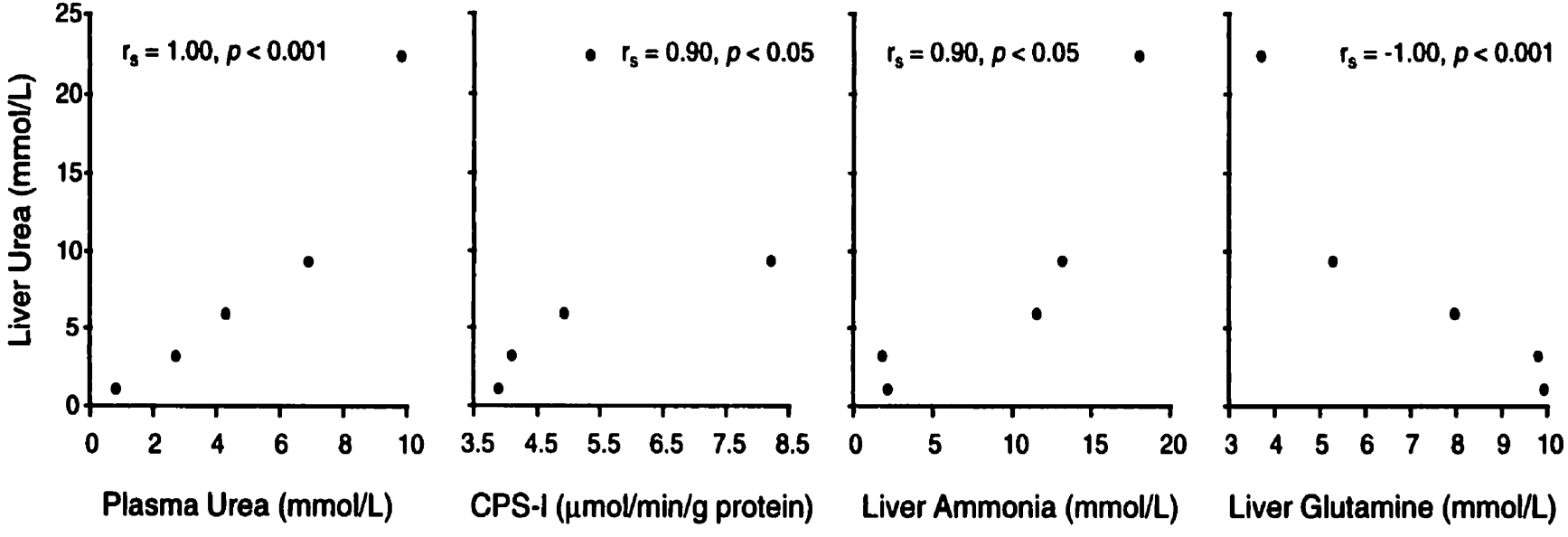


Figure 3.3 Pairwise associations between the liver urea concentration and the concentrations of plasma urea and liver ammonia and glutamine, and between the liver urea concentration and the activity of liver carbamoyl phosphate synthetase I (CPS-I), in five adult male polar bears. Associations were determined using Spearman correlation analysis.

concentrations of plasma constituents during feeding and fasting. Pairwise correlations between the concentrations of specific constituents in plasma also were used to draw inferences of particular biochemical events or associations (Table 3.5).

Fasting polar bears had significantly lower concentrations of urea in plasma than did feeding polar bears (Table 3.2). Further, the urea concentration in plasma was closely correlated with that in liver, and the concentrations in both tissues were positively associated with the liver activity of carbamoyl phosphate synthetase I (CPS-I) (Fig. 3.3), the major enzyme controlling urea synthesis (Meijer 1995). Together, these results suggest that urea was synthesized at a slower rate in polar bears during fasting than during feeding. Urea synthesis decreased in black and brown bears during winter dormancy, and reduced urea synthesis has been proposed to be a mechanism by which protein loss is minimized in these species during fasting (Nelson et al. 1975, Barboza et al. 1997). In this study, the plasma concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were closely correlated with the plasma urea concentration (Table 3.5), and the liver activity of these transaminases was lower during fasting than during feeding. These results suggest that the rate of transamination in liver, and possibly other tissues, was closely coupled with the rate of urea synthesis in polar bears, and that the lower rate of urea synthesis during fasting occurred in part as a result of lower rates of protein and amino acid catabolism.

Fasting polar bears had significantly greater concentrations of β- and γ-globulins in plasma than did feeding polar bears (Fig. 3.1). A role for globulin proteins in metabolism during fasting has not been described previously. Thus, the physiological significance of this difference is unclear. Because feeding and fasting polar bears were captured in two different geographic localities and represent two distinct polar bear populations, the difference in γ-globulin concentrations could reflect a difference in antigenic stimulation between populations, possibly as a result of differences in disease prevalence. Nevertheless, the fact that the concentration of β-globulin also was higher in fasting than in feeding polar bears, and the fact that wide-based peaks occurred in both the β- and γ-globulin regions from fasting polar bears, indicate that the differences between feeding and fasting bears probably involved numerous proteins possessing electrical properties characteristic of β- and

Table 3.5. Pairwise associations between the concentrations of specific plasma constituents in adult polar bears and the proposed biochemical significance for these associations.[a]

Constituent pairs	*r*	Proposed Biochemical Significance
urea - ALT	0.57	- urea synthesis is positively associated with amino acid catabolism
urea - AST	0.53	- urea synthesis is positively associated with amino acid catabolism
urea - phosphoserine	0.78	- urea synthesis is positively associated with the rate of glycolysis
lysine - arginine	0.85	- arginine is positively influenced by lysine inhibition of arginase
glutamine - alanine	0.67	- a significant proportion of plasma alanine is formed from glutamine
glutamate - ammonia	0.63	- a significant proportion of plasma glutamate is formed from ammonia
glutamate - γ-GT	0.76	- γ-glutamyl cycle activity is positively associated with plasma glutamate

[a] Associations were determined using Pearson correlation analyses of plasma constituents in 39 adult polar bears. All associations were significant at $p < 0.001$.

γ-globulin. Thus, although it may be that globulin proteins play a role in the metabolic response of polar bears to fasting, the data from this study provide no insight as to what that role might be.

Fasting polar bears had higher concentrations of creatinine in plasma than did feeding polar bears (Table 3.2). The plasma creatinine concentration is not significantly affected by nutritional state in many mammals (Duncan et al. 1994), but it has been observed to increase during winter dormancy in black bears (Nelson et al. 1984), and during the period of on-land fasting in polar bears (Ramsay et al. 1991). Because the daily excretion of creatinine in urine is similar from day to day and proportional to muscle mass (Rodwell 2000b), an increase in plasma creatinine concentration during fasting could occur as a consequence of decreased urine output. In black bears, urine excretion has been demonstrated to decrease with the onset of winter dormancy (Brown et al. 1971, Nelson et al. 1975). Unfortunately, there is no published information regarding urine excretion for polar bears during feeding and fasting, aside from a few anecdotes to suggest it may be decreased in denning females (Harington 1968, Watts 1983).

In male polar bears, the plasma concentration of total amino acid nitrogen and the proportion of total amino acid nitrogen in amino acids containing more than one amino group were greater during fasting than feeding (Table 3.2 and Fig. 3.2). A plausible explanation for these results is that amino acids containing a single amino group were preferentially catabolized during fasting, and the amino group was subsequently re-incorporated into amino acids that contained more than one amino group. A consequence of this "selective catabolism" is that the carbon skeletons of some amino acids could be made available as substrate for carbohydrate and lipid biosynthesis while other amino acids served to store nitrogen (particularly glutamine and arginine) precluding its excretion via urea synthesis. This inference is well-supported by data from male polar bears, but less so by data from female bears in which the relevant differences between feeding and fasting were less pronounced.

The strong positive correlation between the plasma concentrations of lysine and arginine in polar bears could be explained as a result of competitive inhibition by lysine of arginase, the enzyme through which arginine is converted to urea and ornithine. Lysine and ornithine are potent inhibitors,

while arginine is a potent stimulant, of arginase activity (Hunter and Downs 1945, Rodwell 2000a). Through its inhibitory effect on arginase, lysine has been proposed to play a key role in regulating the supply of ornithine in the thyroid gland of rats (Matsuzaki et al. 1981). According to the explanation hypothesized for polar bears, nitrogen flux through the ornithine-urea cycle would become progressively inhibited at the arginase reaction as the concentration of lysine in the liver increased. This, in turn, would cause the concentration of arginine to rise in the cytosol of liver cells (hepatocytes) in parallel with the lysine concentration, and these concentration increases in the liver eventually would be reflected in the plasma.

The activity of CPS-I in liver and the concentrations of urea in liver and plasma indicate that the rate of urea synthesis in polar bears was lower during fasting than during feeding (Table 3.2 and Fig. 3.3). The results from the killed bears indicated, however, that only the activities of the mitochondrial enzymes CPS-I and OTC were lower during fasting (Table 3.4). In contrast, the activities of the cytosolic enzymes argininosuccinate synthetase (AS), argininosuccinate lyase (AL), and arginase were either similar (as with AS) or greater (as with AL and arginase) during fasting than during feeding. This apparent lack of coordination in the enzyme activity of polar bears contrasts with the coordinated increases in ornithine-urea cycle enzyme activities that are typical of other mammals in response to fasting (Schimke 1962, Morris 1992). When considered together with the hypothesis of competitive inhibition by lysine of arginase, these results suggest that urea synthesis in fasting polar bears was down-regulated through the CPS-I reaction and inhibited through the arginase reaction, as is illustrated in Figure 3.4. The CPS-I reaction reduced entry of ammonia nitrogen into the ornithine-urea cycle (Meijer 1995), and lysine inhibition of arginase reduced the entry of arginine nitrogen into urea and ornithine. As a result, ammonia nitrogen would be diverted towards the biosynthesis of amino acids rather than urea, and nitrogen from citrulline and aspartate would be used to synthesize arginine. This proposed scheme for nitrogen metabolism in the liver of fasting polar bears (see Fig. 3.4) also provides a plausible explanation as to how nitrogen could be progressively redistributed during fasting toward amino acids that contain multiple amino groups, e.g., glutamine, arginine.

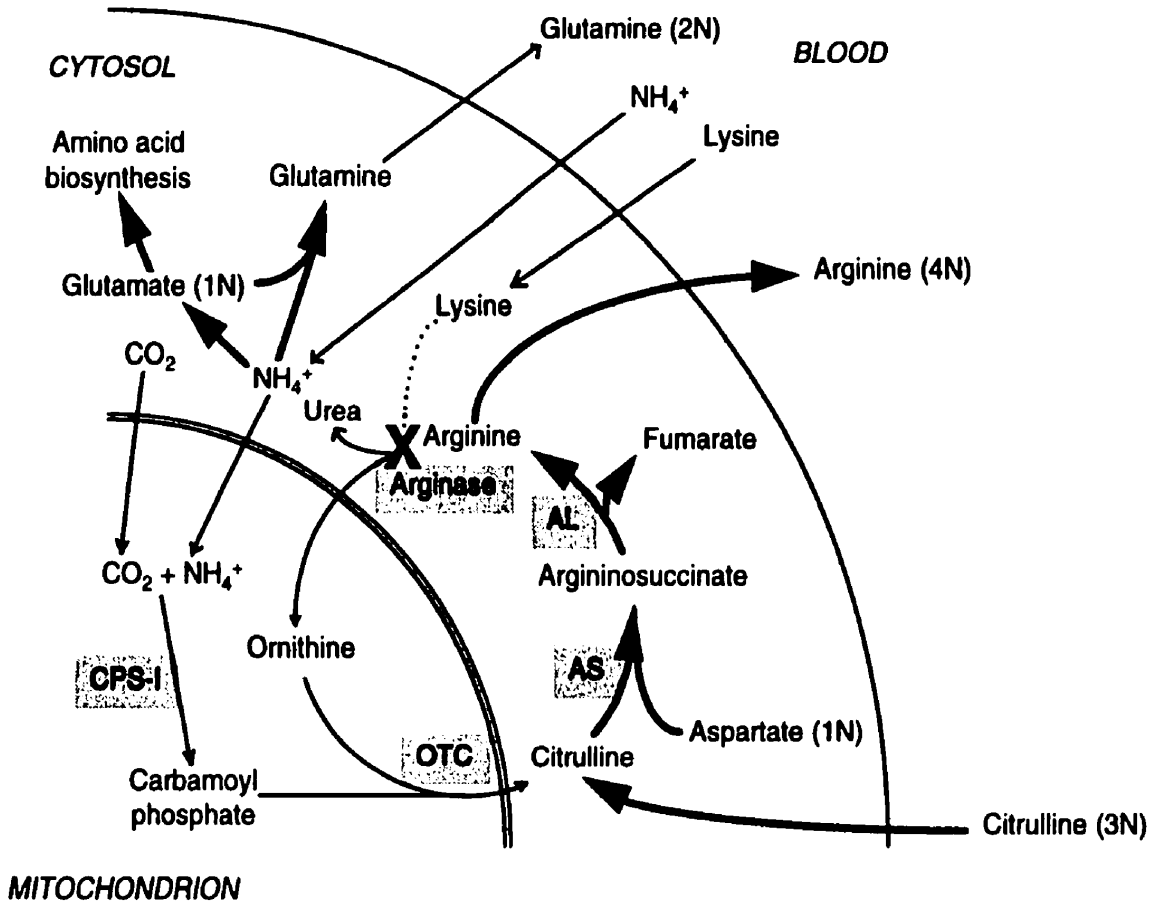


Figure 3.4 Proposed model for nitrogen metabolism in the liver of fasting polar bears. The reactions proposed as favored during fasting are indicated by bold lines and arrows. Inhibition of the conversion of arginine to urea and ornithine is indicated by **X**, and the inhibition of arginase by lysine is indicated by a dotted line. The number of nitrogens within the structure of individual amino acids is presented in round brackets following the name of the amino acid. Ornithine-urea cycle enzymes are carbamoyl phosphate synthetase I (CPS-I), ornithine transcarbamoylase (OTC), argininosuccinate synthetase (AS), argininosuccinate lyase (AL), and arginase.

Rates of glucose production and oxidation appeared to parallel the rate of urea synthesis in polar bears. Fasting bears had lower concentrations of phosphoserine in plasma than did feeding polar bears (Table 3.3), and there was a strong positive correlation between the plasma concentrations of phosphoserine and urea in all bears (Table 3.5). Because phosphoserine is formed from the glycolytic intermediate 3-phosphoglycerate, the plasma concentration of phosphoserine is directly affected by the rate of glycolysis. The finding that the liver PK activity was lower during fasting than during feeding indicated the rate of glycolysis was lower in fasting bears (Table 3.4). Furthermore, the finding in this study that the PK activity ratio was higher (the higher the ratio, the greater the capacity for phosphorylation of PK and the lower the flow of carbon toward gluconeogenesis), and the finding in a concurrent study that the concentration of glycogen in liver was greater in fasting bears than in feeding bears (see Chapter 6), are consistent with lower rates of gluconeogenesis and glycogenolysis during fasting. Together, these results indicate that low rates of glucose production and oxidation in fasting polar bears were associated with a low rate of urea synthesis.

The importance of alanine as a gluconeogenic amino acid and the amount of nitrogen and carbon exchange through the glucose-alanine cycle are likely less significant to fasting polar bears than to fasting humans. In humans, the plasma alanine concentration decreases progressively and significantly during prolonged fasting (> 5 weeks) because the uptake of alanine from plasma by splanchnic tissues and its subsequent use for gluconeogenesis are greater than its release into plasma from muscle (Adibi 1968, Felig et al. 1969). In polar bears, plasma concentrations of alanine were higher during fasting than during feeding (Table 3.3). Fasting bears also had significantly lower concentrations of ALT in plasma (Table 3.2) and less ALT activity in liver (Table 3.4), and therefore presumably less gluconeogenesis from alanine, than did feeding bears. Furthermore, the plasma concentrations of glutamine and alanine were highly correlated in all bears (Table 3.5), and supported the possibility that a significant proportion of the alanine in plasma may have arisen from the catabolism of glutamine in the small intestine (Jungas et al. 1992), instead of through the release of alanine from muscle.

Fasting polar bears had a higher concentration of 3-methylhistidine and a lower concentration of hydroxyproline in plasma than did feeding polar bears (Table 3.3). Because the liver results did not provide additional information regarding the origin or fate of these particular amino acids, suggestions regarding the physiological significance of plasma concentrations during feeding and fasting are largely speculative. 3-Methylhistidine is released from muscle into plasma during the intracellular breakdown of actin and myosin, and the urinary output of this amino acid has been used as an index of the rate of myofibrillar protein breakdown in the musculature of humans (Giesecke et al. 1989). High concentrations of 3-methylhistidine in the plasma of fasting polar bears were unlikely to result from an increased rate of myofibrillar protein breakdown as evidence has been provided to suggest protein and amino acid catabolism was less during fasting than during feeding. Instead, as was suggested with the plasma creatinine concentration, the 3-methylhistidine concentration may have been higher during fasting as a consequence of decreased urine output.

Hydroxyproline is formed from the hydroxylation of peptide-bound proline in collagen, and its appearance in plasma has been used as an index of bone metabolism (specifically, the resorption of calcium) in humans (Minisola et al. 1985) and domestic cattle (Goff and Horst 1997). If the plasma concentration of this amino acid is similarly sensitive to bone metabolism in polar bears, then the lower plasma concentrations of hydroxyproline in fasting relative to feeding bears may reflect a lower rate of bone metabolism during fasting.

3.4.3 Differences between females and males

Plasma urea concentration was low in both sexes during fasting (Table 3.2). Thus, urea synthesis probably occurred at similarly low rates in females and males during fasting irrespective of other differences in the plasma constituents. Many of the differences measured between sexes may reflect differences in protein and amino acid metabolism, but overall nitrogen conservation was similar in females and males.

A general explanation for many of the differences in the concentrations of plasma constituents between females and males may be found in the differences in the timing of maximal

nitrogen requirement relative to availability of food. For females, the requirement for nitrogen is most intense during summer, fall, and winter when food is less available or not available at all. Females that are accompanied by cubs lactate throughout the fasting period (Arnould and Ramsay 1994) and, therefore, must catabolize their muscle protein and amino acids to provide the nitrogen required to produce milk protein. Solitary adult females are often pregnant when food is not available (Derocher et al. 1992) and, therefore, must catabolize their muscle protein and amino acids to provide the nitrogen required to form and maintain a conceptus throughout the period of gestation, i.e., conception during April or May, followed by delayed implantation in October or November, and parturition in December or January (Lønø 1972, Ramsay and Stirling 1988, Atkinson and Ramsay 1995). Thus, for most females, the requirement for nitrogen is maximal when food is unavailable, and nitrogen must be provided through the catabolism of stored body protein. In contrast, the requirement for nitrogen in adult males is probably greatest in association with mating activity during spring, a time when food (i.e., seal pups) is abundant. It is at this time that serum testosterone concentrations attain their annual peak (Palmer et al. 1988). High circulating concentrations of testosterone affect protein metabolism by increasing the rate of protein synthesis while simultaneously lowering the rate of protein catabolism (Ganong 1995). Thus, for most males, the requirement for nitrogen is maximal when food and, therefore, diet-derived nitrogen is available. Males may fast for brief periods when seeking opportunities to mate, but their high serum concentrations of testosterone protect their stored body protein from net catabolism.

Some significant differences between female and male polar bears in this study conform well to the above general model. The plasma concentrations of total amino acid, total amino acid nitrogen, and nutritionally non-essential amino acid were lower in fasting females than in feeding females and, conversely, lower in feeding males than in fasting males (Table 3.2). Low concentrations in fasting females could occur as a result of an increased requirement of plasma amino acids for the synthesis of milk (in the case of seven lactating females captured during summer-fall), or for the development and maintenance of a conceptus (in the case of four solitary females captured during summer-fall). This inference is supported by the observation that the concentration

of γ-glutamyltransferase (γ-GT) was higher in the plasma of fasting females than in that of feeding females or fasting males. γ-Glutamyltransferase (also called γ-glutamyl transpeptidase) is an enzyme of the γ-glutamyl cycle; a biochemical pathway of major importance in the metabolism of amino acids and their translocation between tissues, and in the synthesis of glutathione. Under pathological conditions, a marked increase in the concentration of γ-GT in the plasma is most commonly attributed to the leakage of γ-GT from damaged liver cells (Duncan et al. 1994, Rodwell and Kennelly 2000). In normal health, however, γ-GT can leak into plasma from other tissues because γ-GT occurs as a membrane-bound enzyme in a variety of tissues that include liver, heart, kidney, skeletal muscle, and tissues of the testicles, mammary glands, fetus, and placenta (Rollins et al. 1981, Puente et al. 1982, Castellon 1994, Courtay et al. 1994, Hanigan and Frierson 1996, Leeuwenburgh et al. 1997). Amino acids have been shown to be translocated from the maternal tissues of rats through the γ-glutamyl cycle to be used by placental and fetal tissues and the lactating mammary gland during pregnancy and lactation (Viña et al. 1989). Thus, the higher plasma concentration of γ-GT in fasting females than in fasting males could result from higher γ-glutamyl cycle activity in females with the subsequent leakage of γ-GT into plasma from the tissues of the mammary gland or fetus and placenta, and the removal of amino acids (specifically glutamine, cystine, and other neutral amino acids) from the plasma by these tissues.

The lower concentrations of total amino acid, total amino acid nitrogen, and nutritionally non-essential amino acid in feeding males (relative to fasting males) could occur as the net result of a high rate of protein synthesis and a low rate of protein catabolism; both effects being induced through high circulating concentrations of serum testosterone (Palmer et al. 1988, Ganong 1995). Plasma concentrations of ALT, AST, γ-GT, and ammonia were greater in feeding males than in feeding females, but the plasma urea concentrations did not differ significantly between sexes. This suggests that the loss of nitrogen through urea excretion was similar in both sexes, and that transamination was greater in males as a result of anabolic processes instead of catabolic processes. The higher concentration of γ-GT could reflect higher rates of amino acid metabolism and translocation through the γ-glutamyl cycle in testicles and skeletal muscle. The higher concentration

of ammonia could have occurred because ammonia nitrogen was being preferentially directed into amino acid biosynthesis instead of into storage as glutamine.

Glutamine was the most abundant free amino acid in the plasma of polar bears (Table 3.3). Nevertheless, there were differences between females and males in that plasma concentrations of glutamine were higher in feeding females than in fasting females and, conversely, higher in fasting males than in feeding males. Plasma glutamine plays an important role in preserving amino acid balance by conserving nitrogen and reducing its excretion as urea (Heitmann and Bergman 1980, Cersosimo et al. 1986). The time when glutamine concentrations were highest in female bears coincided with a time when food was available, net protein catabolism was minimal, and excess nitrogen could be effectively conserved in plasma as glutamine. The time when glutamine concentrations were highest in male bears coincided with a time when food was unavailable, net protein catabolism was maximal, and nitrogen arising from protein catabolism could be effectively conserved in plasma as glutamine.

The pattern of differences between female and male polar bears in plasma glutamate concentration was opposite to the pattern observed with the plasma glutamine concentration (Table 3.3). Plasma concentrations of glutamate were higher in fasting females than in feeding females and, conversely, higher in feeding males than in fasting males. Thus, in contrast to glutamine, plasma glutamate concentrations were highest in female and male bears at the time when their nitrogen requirements were highest. An explanation for these observations is that conservation of nitrogen in glutamine was less when nitrogen requirements were high, and ammonia nitrogen was preferentially directed toward glutamate formation with subsequent biosynthesis of other amino acids and glutathione formation. This hypothesis is supported, in part, by the highly significant and positive correlations between the plasma concentrations of glutamate and ammonia, and between glutamate and γ-GT (Table 3.5).

3.4.4 Association with body condition

In this study, body condition was defined as the abundance of potential metabolic energy stored in an animal's tissues (primarily adipose tissue and skeletal muscle) relative to its body size. The body condition index (BCI) was developed to provide an estimate of the body condition of individual polar bears relative to other bears in a sample population of 1072 animals (see Chapter 2). Although the BCI is an index without units, it can be used in conjunction with the body length of a bear to predict its mass of adipose tissue, skeletal muscle, or both tissues. The range of BCI values is distributed normally around a mean value of zero, with positive values indicating a larger availability of potential metabolic energy and negative values indicating a smaller availability of potential metabolic energy, relative to the mean body condition of the sample population.

The BCI was strongly correlated with many of the plasma constituents that reflected protein and amino acid metabolism in polar bears. Furthermore, the effect of large body lipid stores (positive BCI value) and fasting on the plasma concentrations of many constituents were similar. These observations provide strong support to indicate that the underlying energy metabolism in both states (fasting and fat) is one in which lipid is the predominant fuel for energy and nitrogen is conserved. These results also support previous studies that have shown the rate of protein catabolism during prolonged fasting in some species, including polar bears, is inversely associated with the magnitude of body lipid stores (Forbes and Drenick 1979, Cherel et al. 1992, Atkinson et al. 1996a).

3.4.5 Effect of anesthesia

The anesthetic drug Telazol® has not been investigated for its effect on the concentrations of plasma constituents associated with protein and amino acid metabolism in mammals. Thus, it is not possible to clearly determine whether or not Telazol® affected the measured variables. Nevertheless, the uniform treatment of all bears with Telazol® makes any effects of this drug a fairly constant factor in the study which should not confound the analysis of the effects of other variables. Furthermore, the observation that the dosage of Telazol® administered to polar bears in this study did

not correlate with the concentrations of any plasma constituents suggests that the effects of Telazol® were minimal.

3.4.6 Conclusions

Nutritional state, sex, and body condition affected the concentrations and activity of many of the plasma and liver constituents associated with protein and amino acid metabolism in polar bears.

Protein and amino acid catabolism and urea synthesis were significantly lower during fasting. This may represent one mechanism by which polar bears are able to spare body protein during prolonged fasts. Nevertheless, the sparing of protein did not appear to be absolute and some amino acids were likely catabolized to provide substrates for carbohydrate and lipid biosynthesis. Much of the nitrogen from protein and amino acid catabolism was not irreversibly lost through urea excretion, but was conserved through the preferential catabolism of amino acids carrying a single nitrogen and the re-incorporation of this nitrogen into amino acids containing multiple nitrogens (e.g., glutamine and arginine). The diverting of amino acid nitrogen away from urea formation is facilitated in part by an imbalance in the enzyme activity of the ornithine-urea cycle at two points. First, ammonia nitrogen is inhibited from entering the ornithine-urea cycle at the CPS-I reaction and is, therefore, diverted into storage as glutamine or into amino acid biosynthesis through glutamate. Second, the amino nitrogen in citrulline and aspartate is combined (via the AS and AL reactions) eventually to form arginine, while arginine is inhibited from further entering the ornithine-urea cycle because of competitive inhibition of the arginase reaction by lysine (Fig. 3.4).

Many of the differences in the concentrations of plasma constituents between female and male polar bears reflect differences in protein and amino acid metabolism, but overall nitrogen conservation was similar in both sexes. Differences in protein and amino acid metabolism between female and male bears are explained by differences in the timing of maximal nitrogen requirement relative to availability of food. Nitrogen requirements (for lactation or pregnancy) in female bears are maximal when food is unavailable, and nitrogen must be provided through the catabolism of

stored body protein. Nitrogen requirements (for mating activity) in male bears are maximal when food and, therefore, diet-derived nitrogen is available.

The effects of large body lipid stores (positive BCI value) and fasting on the concentrations of many plasma constituents were similar, and indicate that the underlying energy metabolism in both states (fasting and fat) is one in which lipid is the predominant fuel for energy and nitrogen is conserved.

4. ASPECTS OF LIPID METABOLISM IN FREE-RANGING ADULT POLAR BEARS

4.1 Introduction

High fat and low carbohydrate diet, seasonal obesity, and prolonged fasting are the usual circannual dietary habits of the polar bear (*Ursus maritimus*). These habits suggest that the survival of polar bears is contingent upon their ability to closely regulate adipose accretion and lipid metabolism. However, aside from observations that the plasma of polar bears contains higher concentrations of lipids than occur in the plasma of healthy humans (Ferguson and Folk 1971, Kaduce et al. 1981), the metabolism of lipids by this species has not been studied.

In this study, plasma concentrations of lipids, lipid precursors and metabolites, total carnitine, and carnitine fractions were measured in free-ranging polar bears captured throughout the year to determine the association of these plasma constituents with nutritional state (feeding versus fasting), sex (female versus male), and body condition (lean versus fat). Plasma glycerol kinetics also were measured in polar bears following intravenous administration of ^{13}C-glycerol to compare rates of lipolysis among bears of different nutritional state, sex, and body condition.

4.2 Materials and methods

Eighty-two free-ranging, adult polar bears were captured from April 1995 to November 1996. All bears were located by helicopter and chemically immobilized with Telazol® (8 to 12 mg/kg intramuscularly) using remote injection equipment (Stirling et al. 1989). Twenty-four bears were captured during spring (April and May) while they were hunting seals on arctic sea-ice near Resolute Bay, Nunavut, Canada (74°00' to 76°00'N and 93°00' to 100°50'W). Fifty-eight adult polar bears were captured during summer, fall, and winter (July to November, and March) while they were fasting on land along the west coast of Hudson Bay near Churchill, Manitoba, Canada (57°00'

to 58°50'N and 92°25' to 94°15'W). The polar bears captured during March were comprised of ten adult females accompanied by new-born cubs that had emerged recently (within days) from their snow dens after 8 to 9 months of fasting that included pregnancy and early lactation.

Bears were weighed (± 0.5 kg) with an electronic load scale while suspended in a sling. Body length was measured by positioning bears in sternal recumbency and measuring the straight-line distance (± 0.5 cm) between the distal tips of nose and tail. Body condition of captured bears was quantified by a body condition index (BCI) based on the relationship between body mass and length (see Chapter 2). Ages of bears previously captured were obtained from a national database (Canadian Wildlife Service, Edmonton, Alberta). The ages of bears not previously captured were determined by extracting, sectioning, staining, and counting cementum annuli on one first premolar tooth (Stoneberg and Jonkel 1966). Bears 5 years of age or older were classified as adults (Lunn et al. 1997).

Twenty milliliters of blood was collected from the jugular vein into Vacutainer® tubes containing sodium heparin (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). Blood samples were cooled immediately following collection and were centrifuged within 12 hours to collect and freeze plasma. Plasma concentrations of triacylglycerol, non-esterified fatty acid (NEFA), glycerol, total cholesterol, unesterified cholesterol, and high density lipoprotein (HDL-) cholesterol were measured by Molecular Biology Institute Lipid and Lipoprotein Laboratories (UCLA, California) using the methods of Burstein et al. (1970), Warnick (1986), and Puppione and Charungundla (1994). Plasma phospholipid was measured at the Hormel Institute, University of Minnesota, using the methods of Holman (1989). Total carnitine and free carnitine (FC) were measured by radioenzymatic analysis at Metabolic Analysis Labs, Inc. (Madison, WI) using the method of Parvin and Pande (1977). Acyl-carnitine (AC) was calculated as the difference in plasma concentration between total and free carnitine. Plasma β-hydroxybutyrate was measured by enzymatic analysis at the University of Illinois Veterinary Medical Clinic, and acetoacetate was measured by the nitroprusside reaction at the Clinical Pathology Laboratory, Western College of Veterinary Medicine, University of Saskatchewan.

Plasma glycerol kinetics were measured in 36 polar bears by administering glycerol, in which all three carbons were labeled with the stable isotope ^{13}C (U-$^{13}C_3$-glycerol, 99%; Cambridge Isotope Laboratories, Inc., Andover, MA), by bolus injection via a jugular catheter (dosage range: 2.4-5.0 μmol/kg). A baseline blood sample was drawn immediately prior to ^{13}C-glycerol administration and samples were taken at 5, 10, 15, 30, 60, 90, 120, and 150 minutes following ^{13}C-glycerol administration. The enrichment of plasma glycerol (tracee) with ^{13}C-glycerol (tracer) was measured as the tracer:tracee ratio by the Metabolism Unit, Shriners Burns Institute (Galveston, TX), using the methods described in Klein et al. (1986).

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, USA). Two-way analysis of variance (ANOVA) was used to determine differences in plasma lipids, lipid precursors and metabolites, and total carnitine and carnitine fractions among nutritional states (feeding, fasting, and fasting that included pregnancy and early lactation), and between sexes (Zar 1996). The body condition index (BCI) and drug dosage (mg/kg) were included as covariates. Tukey's Honestly Significant Difference test was used to make multiple comparisons among means (Zar 1996). Nonlinear least squares estimation was used to calculate an exponential model of the form $y(t) = \sum A_i e^{-\lambda i}$ (tracer:tracee ratio) to describe the disappearance of ^{13}C-glycerol from plasma (Draper and Smith 1981). The criterion for best model was a minimum value for the residual sum of squares. Kinetic parameters for glycerol were calculated by noncompartmental analysis (Wolfe 1992) as follows:

$$\text{Rate of appearance } (R_a) \text{ (μmol/kg per min)} = \text{Tracer dose} \div \sum(A_i/\lambda_i) \qquad \text{(Eqn. 4.1)}$$

where R_a refers to the rate of appearance of glycerol in plasma (Wolfe 1992), and where A_i and λ_i are the macroparameters for the nonlinear model (Draper and Smith 1981);

$$\text{Rate of clearance } (R_c) \text{ (ml/kg per min)} = R_a \div \text{glycerol plasma concentration} \qquad \text{(Eqn. 4.2)}$$

where R_c refers to volume of plasma completely cleared of glycerol in 1 minute (Wolfe 1992); and

$$\text{Half-life } (T_{½}) \text{ for } ^{13}\text{C-glycerol (min)} = (\ln 2) \div \Sigma\lambda_i \qquad \text{(Eqn. 4.3)}$$

where $T_{½}$ refers to the time required for the injected ^{13}C-glycerol to reach one-half its initial concentration in plasma (Wolfe 1992).

Values for R_a were estimated on the basis of total body mass (μmol/kg per min), fat mass (μmol/kg FM per min), and lean body mass (μmol/kg LBM per min). Values for R_c were estimated on the basis of total body mass (ml/kg per min) and LBM (ml/kg LBM per min). FM was estimated using a regression model with body length and the BCI value as the predictor variables (see Chapter 2), and LBM was calculated by subtracting FM from the total body mass. Statistical assumptions of normality, independence, and homogeneity of variance were maintained by data transformation (natural log or square root), as necessary. For all statistical tests, significance was assigned when the probability of a Type I error was equal to or less than 0.05. All results are reported as mean ± standard error (SE).

4.3 Results

4.3.1 Association with nutritional state and sex

Differences in nutritional state (feeding versus fasting) had no significant effect on plasma concentrations of non-esterified fatty acid (NEFA), glycerol, acetoacetate, or β-hydroxybutyrate, or the NEFA:glycerol ratio (Table 4.1). However, the sex of bear did have an effect on plasma NEFA concentration; values for females were significantly higher than values for males ($F = 6.38$, $p < 0.05$, $n = 72$). Acetoacetate concentration was below the level of detection (< 196 μmol/L) in all bears and β-hydroxybutyrate concentration never exceeded 291μmol/L.

The disappearance of ^{13}C-glycerol from plasma fit best an exponential model that was comprised of two exponents, $y(t) = A_1e^{-\lambda 1} + A_2e^{-\lambda 2}$ (Fig. 4.1). The residual sum of squares as a proportion of the corrected sum of squares, for the 36 bears, was equal to 0.05±0.02. There were no

Table 4.1 Plasma concentrations of lipids, lipid precursors and metabolites, and total carnitine in free-ranging adult polar bears captured during feeding (spring) and fasting (summer-fall), and during fasting that included pregnancy and early lactation (winter).[a]

Plasma constituent or ratio	Feeding (spring)		Fasting (summer-fall)		Fasting+[b] (winter)	Factor or Covariate			
	female (n = 12)	male (n = 12)	female (n = 16)	male (n = 32)	female (n = 10)	nutritional state	sex	nutritional state × sex	BCI
total lipid (g/L)	9.7±0.28 (8.2-11.6)	8.0±0.64 (4.8-10.5)	7.8±0.35 (5.0-9.2)	7.8±0.20 (5.1-11.1)	9.5±0.63 (8.2-11.8)	ns	*	***	***
total cholesterol (mmol/L)	10.8±0.43 (7.6-12.4)	8.1±0.62 (4.4-11.5)	8.3±0.46 (3.9-10.5)	8.3±0.23 (4.4-11.2)	11.0±1.00 (4.9-16.5)	ns	**	***	***
phospholipid (g/L)	3.8±0.17 (3.1-5.1)	2.8±0.16 (1.4-3.5)	2.6±0.12 (1.6-3.3)	2.5±0.06 (1.6-3.8)	3.8±0.20 (3.3-4.5)	***	***	***	***
triacylglycerol (mmol/L)	2.2±0.14 (1.5-3.1)	1.4±0.31 (0.1-3.5)	2.1±0.21 (1.1-3.3)	2.5±0.12 (1.0-4.3)	1.5±0.18 (0.6-2.5)	***	ns	***	ns
non-esterified fatty acid (NEFA; mmol/L)	1.6±0.20 (0.6-2.8)	1.2±0.14 (0.5-1.8)	1.8±0.23 (0.6-3.0)	1.2±0.14 (0.2-3.2)	1.5±0.31 (0.3-2.8)	ns	*	ns	ns
glycerol (mmol/L)	3.8±0.93 (0.9-9.8)	3.0±0.50 (0.8-6.2)	2.5±0.35 (1.3-5.6)	2.0±0.25 (0.3-7.1)	2.1±0.51 (0.5-4.7)	ns	ns	ns	ns
NEFA:glycerol ratio	0.67±0.14 (0.08-1.92)	0.52±0.10 (0.17-1.46)	0.79±0.09 (0.37-1.58)	0.65±0.06 (0.17-1.85)	0.77±0.11 (0.42-1.59)	ns	ns	ns	ns
acetoacetate (µmol/L)[c]	< 196	< 196	< 196	< 196	< 196	ns	ns	ns	ns
β-hydroxybutyrate (µmol/L)	162±64.7 (97-291)	97±25.1 (0-194)	125±40.8 (0-291)	127±20.2 (0-291)	136±23.8 (97-194)	ns	ns	ns	ns
total carnitine (µmol/L)	44±3.1 (36-73)	57±5.7 (37-103)	51±5.6 (23-93)	48±2.0 (20-68)	110±7.6 (66-149)	***	ns	*	***

[a] Values reported as mean ± SE with range presented in parentheses. Statistical comparison made by two-way ANOVA with nutritional state and sex as the factors, nutritional state × sex as the interaction, and the Body Condition Index (BCI) as the covariate. Significance indicated by "ns" for non-significance, * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

[b] Adult female bears captured during winter that were accompanied by new-born cubs, and that had emerged recently (within days) from their snow dens.

[c] Acetoacetate concentrations were below the level of detection (< 196 µmol/L) for all samples.

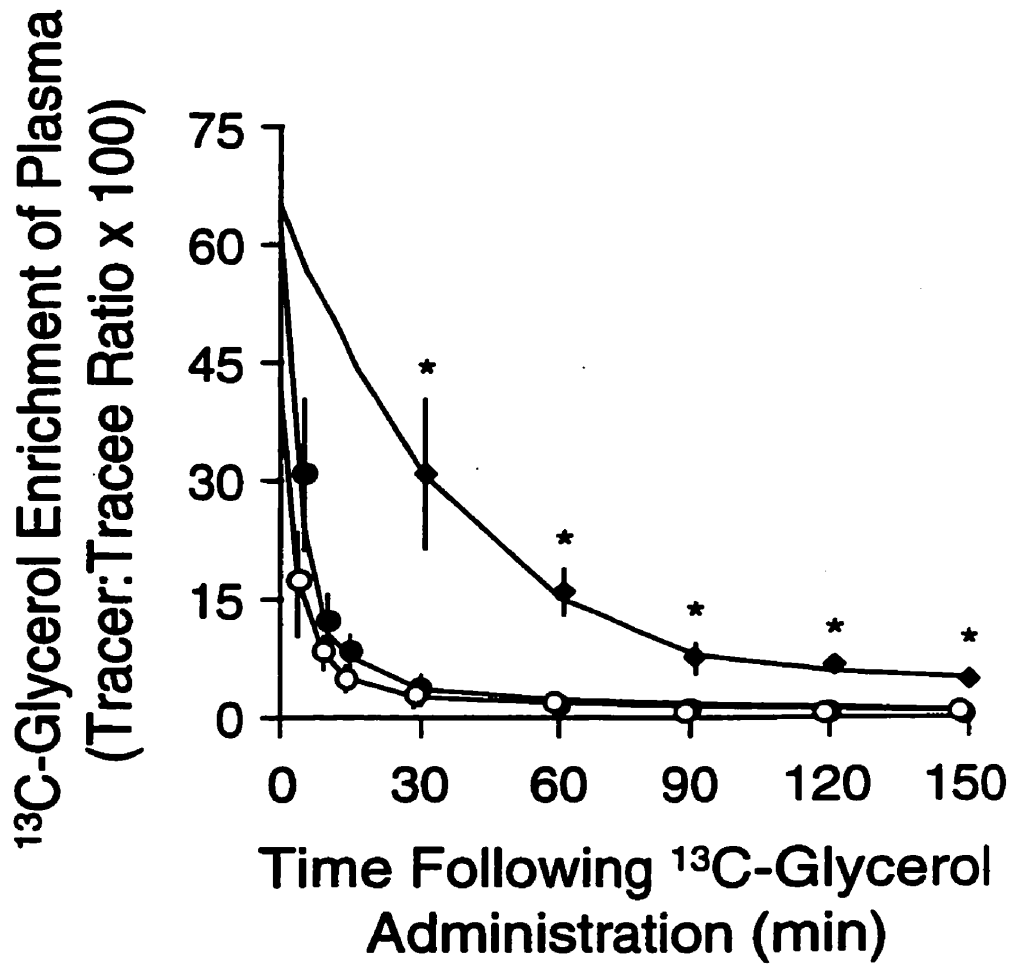


Figure 4.1 The disappearance of ^{13}C-glycerol from the plasma of polar bears. Mean ± standard error bars are presented for 13 feeding bears (○), 18 fasting bears (●), and five fasting female bears captured during winter (◆). Females captured during winter were accompanied by new-born cubs and had recently (within days) emerged from a snow den. Lines represent disappearance curves for ^{13}C-glycerol that were calculated using the method of nonlinear least squares regression. The asterisk indicates female bears captured during winter had a significantly ($p \leq 0.05$) greater tracer:tracee ratio than other bears at the particular sampling time.

significant differences between feeding bears and fasting bears captured during summer-fall in their mean tracer:tracee ratio at each sampling time following ^{13}C-glycerol administration. However, although the sampling of female bears captured during winter did not begin until 30 minutes following ^{13}C-glycerol administration, their mean tracer:tracee ratio at this time, as well as at all sampling times afterward, was significantly greater than that of bears captured during other times of the year. The calculated rates of appearance (R_a) and clearance (R_c) of plasma glycerol varied greatly among individual bears irrespective of nutritional state, but overall the mean R_a and R_c values were significantly lower ($F = 2.75$ to 5.14, $p < 0.05$) in fasting females captured during winter than in feeding or fasting bears captured at other times of year (Table 4.2). Furthermore, the half-life calculated for the disappearance of ^{13}C-glycerol from plasma was significantly greater ($F = 6.53$, $p < 0.001$) in fasting females captured during winter than in polar bears captured at other times of year.

Differences in nutritional state, sex, or in the interaction of nutritional state and sex did have a significant effect on plasma concentrations of total lipid, total cholesterol, phospholipid, triacylglycerol, and total carnitine (Table 4.1). Among female polar bears, plasma total lipid, total cholesterol, and phospholipid values were greater in feeding bears, and in fasting bears captured during winter, than in fasting bears captured during summer-fall (one-way ANOVA: $F = 6.79$ to 24.12, $p < 0.01$, $n = 38$). Feeding females and fasting females captured during summer-fall had higher plasma triacylglycerol values than did fasting females captured during winter ($F = 4.26$, $p < 0.05$). Conversely, fasting females captured during winter had higher total carnitine values than did feeding and fasting females captured at other times of the year ($F = 35.75$, $p < 0.001$). Among male polar bears, plasma concentrations of total lipid, total cholesterol, and phospholipid did not differ significantly between feeding and fasting bears ($t = 0.26$ to 1.75, $p \geq 0.10$, $n = 44$). However, fasting males had significantly higher triacylglycerol ($t = 3.11$, $p < 0.01$) and lower total carnitine values ($t = 1.99$, $p \leq 0.05$) than did feeding males. Among feeding bears, females had significantly higher plasma concentrations of total lipid, total cholesterol, phospholipid, and triacylglycerol than did males ($t = 2.22$ to 4.35, $p < 0.05$, $n = 24$). Conversely, feeding males had significantly higher plasma concentrations of total carnitine than did feeding females ($t = 2.06$, $p \leq 0.05$). There were no

Table 4.2 Plasma concentration and kinetic parameters of glycerol in free-ranging adult polar bears captured during feeding (spring) and fasting (summer-fall), and during fasting that included pregnancy and early lactation (winter).[a]

Constituent or kinetic parameter	Nutritional State			Factor or Covariate			
	feeding (n = 13)	fasting (n = 18)	fasting+[b] (n = 5)	nutritional state	sex	nutritional state × sex	BCI
plasma glycerol (mmol/L)	2.7±0.69 (0.8-9.8)	2.0±0.32 (0.5-5.6)	3.1±0.88 (0.8-4.7)	ns	ns	ns	ns
rate of appearance: R_a (µmol/kg per min)	0.783±0.219 (0.002-2.471)	0.560±0.195 (0.001-2.619)	0.024±0.008 (0.0003-0.034)	*	ns	ns	ns
- R_a (µmol/kg FM per min)	6.513±1.745 (0.016-19.591)	1.874±0.704 (0.003-9.914)	0.122±0.060 (0.002-0.277)	*	ns	ns	ns
- R_a (µmol/kg LBM per min)	0.907±0.254 (0.003-2.828)	0.558±0.257 (0.002-3.558)	0.021±0.010 (0.0004-0.039)	*	ns	ns	ns
rate of clearance: R_c (ml/kg per min)	0.363±0.093 (0.001-0.878)	0.311±0.095 (0.0003-1.206)	0.007±0.003 (0.0001-0.012)	*	ns	ns	ns
- R_c (ml/kg LBM per min)	0.415±0.105 (0.002-0.974)	0.248±0.072 (0.0005-1.024)	0.006±0.003 (0.0001-0.014)	*	ns	ns	ns
half-life for ^{13}C-glycerol: $T_{½}$ (min)	3.4±0.65 (0.3-7.4)	3.7±0.45 (0.4-7.9)	11.7±3.52 (7.4-18.6)	***	ns	ns	ns

[a] Values reported as mean ± SE with range presented in parentheses. Statistical comparison made by two-way ANOVA with nutritional state and sex as the factors, nutritional state × sex as the interaction, and the Body Condition Index (BCI) as the covariate. R_a and R_c values were transformed to their square root prior to analysis, and are expressed per unit body mass (µmol/kg per min or ml/kg per min), per unit fat mass (µmol/kg FM per min), and per unit lean body mass (µmol/kg LBM per min or ml/kg LBM per min). Significance indicated by "ns" for non-significance, * for $p \leq 0.05$, and *** for $p \leq 0.001$.

[b] Adult female bears captured during winter that were accompanied by new-born cubs, and that had emerged recently (within days) from their snow dens.

significant differences between sexes in the concentrations of plasma constituents among fasting bears captured during summer-fall.

Differences in nutritional state, and in the interaction between nutritional state and sex, had a significant effect on the plasma concentrations of acyl-carnitine (AC) and free carnitine (FC) (Fig. 4.2). Among female polar bears, AC and FC values were twice as high in fasting females captured during winter as in females captured at other times of the year ($F = 9.65$ to 35.36, $p < 0.001$, $n = 38$). Among males, the plasma AC concentration was significantly higher in feeding males than in fasting males ($t = 2.21$, $p < 0.05$, $n = 44$), and the plasma FC concentration did not differ significantly between feeding and fasting males ($t = 1.63$, $p = 0.11$). The AC:FC ratio was higher in fasting females than in fasting males ($t = 2.31$, $p < 0.05$, $n = 48$).

The interaction of nutritional state and sex had a significant effect on the plasma concentration of unesterified cholesterol ($F = 12.17$, $p < 0.001$); the concentration was higher in feeding females than in fasting females and, conversely, lower in feeding males than in fasting males (Fig. 4.3). The plasma concentration of high density lipoprotein (HDL-) cholesterol was significantly higher in fasting females captured during winter than in females captured at other times of the year (one-way ANOVA: $F = 4.96$, $p < 0.05$, $n = 38$), and higher in feeding males than in fasting males ($t = 2.12$, $p < 0.05$, $n = 44$). Differences in nutritional state did not have a significant effect on the total cholesterol:HDL-cholesterol ratio in females, but did have a significant effect in males; the ratio was higher in fasting males than in feeding males ($t = 2.09$, $p < 0.05$).

4.3.2 Association with body condition and drug dosage

The body condition index (BCI) covaried significantly with the plasma concentrations of total lipid, total cholesterol, unesterified cholesterol, phospholipid, total carnitine, acyl-carnitine (AC), and free carnitine (FC) (Table 4.1). Furthermore, the correlation of the BCI with the plasma values of each of these constituents was negative, the plasma concentrations of each constituent being lower in bears with higher amounts of body fat (total lipid: $r = -0.41$, $p < 0.001$, $n = 82$; total cholesterol: $r = -0.32$, $p < 0.01$; unesterified cholesterol: $r = -0.32$, $p < 0.01$; phospholipid: $r = -0.37$,

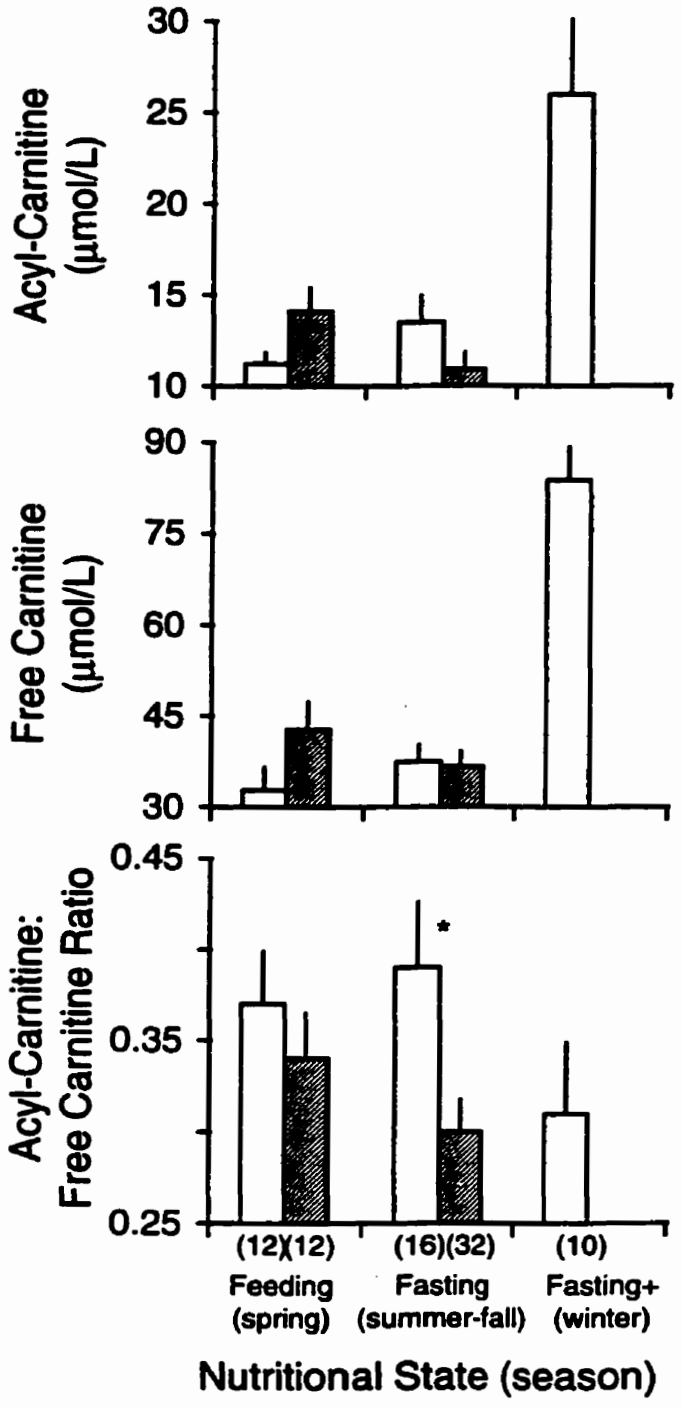


Figure 4.2 Plasma concentrations of acyl-carnitine (AC), free carnitine (FC), and the AC:FC ratio in feeding and fasting polar bears. Values are presented as a bar for the mean plus a vertical line for the standard error. The bars represent females (□) and males (▩). Females captured during winter were accompanied by new-born cubs and had recently (within days) emerged from a snow den. Number of bears captured by season and sex is presented in parentheses. The asterisk indicates a significant difference ($p \leq 0.05$) in mean values between female and male bears during a particular season.

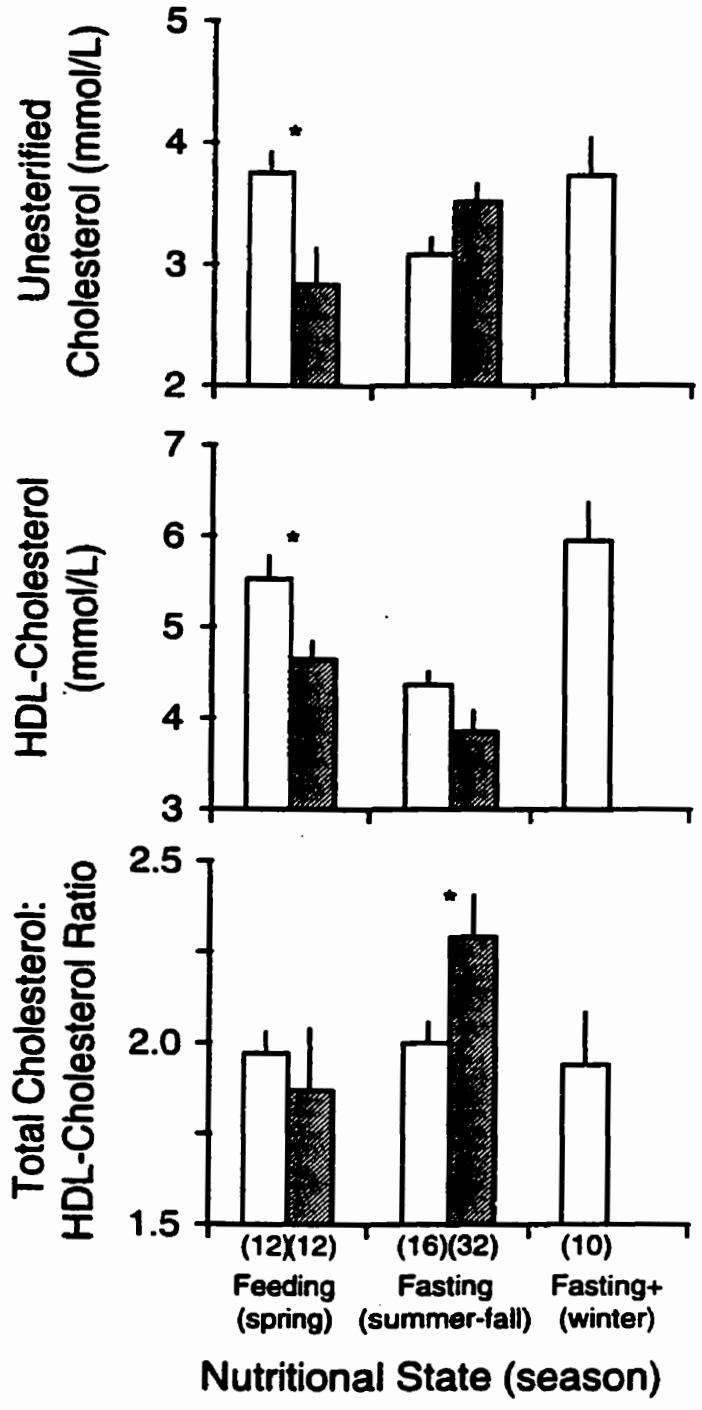


Figure 4.3 Plasma concentrations of unesterified cholesterol, high density lipoprotein (HDL-) cholesterol, and the total cholesterol:HDL cholesterol ratio in feeding and fasting polar bears. Values are presented as a bar for the mean plus a vertical line for the standard error. The bars represent females (□) and males (■). Females captured during winter were accompanied by new-born cubs and had recently (within days) emerged from a snow den. Number of bears captured by season and sex is presented in parentheses. The asterisk indicates a significant difference ($p \leq 0.05$) in mean values between female and male bears during a particular season.

$p < 0.001$; total carnitine: $r = -0.40$, $p < 0.001$; AC: $r = -0.28$, $p < 0.05$; FC: $r = -0.40$, $p < 0.001$).

There were no significant correlations of drug dosage with the concentrations of plasma constituents or with the kinetic parameters for plasma glycerol.

4.4 Discussion

4.4.1 Association with nutritional state and sex

In the polar bears of this study, plasma concentrations of non-esterified fatty acid (NEFA), glycerol, acetoacetate, and β-hydroxybutyrate, and the NEFA:glycerol ratio were not affected by differences in nutritional state (Table 4.1). This contrasts with other mammals (for example, humans - Cahill et al. 1966, Owen et al. 1998; hibernating rodents - Krilowicz 1985, Yeh et al. 1995; black bears, *Ursus americanus* - Ahlquist et al. 1984, Hasan 1997) and suggests that food availability either had little effect on the proportional use of lipid-based (relative to protein- and carbohydrate-based) fuel for energy by polar bears or, if there was an effect, it was not reflected by differences in the plasma concentrations of these lipid metabolites. Results from the investigation of protein and carbohydrate metabolism in polar bears (see Chapters 3 and 6) indicate that protein and amino acid metabolism, and to a lesser extent glucose metabolism, are affected by differences in nutritional state. Thus, it seems unlikely that the proportional use of lipid, protein, and carbohydrate for energy metabolism would not be affected by food availability. Alternatively, for polar bears to balance the synthesis (lipogenesis and ketogenesis), release (lipolysis), and use (oxidation of fatty acids and ketone bodies, gluconeogenesis from glycerol) of lipid metabolites without significant alteration in their plasma concentrations, there must be feedback controls of great precision. This contrasts strongly with the mismatch between fatty acid supply and lipid oxidation that is typical of other mammals during fasting (Hales et al. 1978, Coppack et al. 1994). This apparent ability to control lipid metabolism with great precision may be essential in polar bears which normally consume a diet high in fat and low in carbohydrate.

The rate of appearance (R_a) of plasma glycerol was extremely slow in polar bears (Table 4.2) relative to values reported for other species (for example, humans - $R_a = 2.59 \pm 0.22$ μmol/kg per

min, Klein et al. 1986; dogs - R_a = 1.87±0.15 μmol/kg per min, Previs et al. 1996; goats - R_a = 3.83±0.11 μmol/kg per min, Weber et al. 1993). In many species, the R_a of glycerol is thought to reflect accurately the rate of hydrolysis of triacylglycerol into fatty acids and glycerol (lipolysis) in adipose tissue because, typically, there is little glycerol kinase activity in this tissue (Margolis and Vaughn 1962, Lin 1977). Therefore, glycerol is released quantitatively from adipose tissue into the plasma (Wolfe 1992). However, it appears that the R_a values for glycerol calculated for polar bears in this study were considerably less than the actual rates of lipolysis since the energy which could be made available for lipid oxidation from the calculated R_a values would only provide a small portion of the total energy requirements for an adult polar bear. For example, the highest R_a value for glycerol calculated in this study was 2.62 μmol/kg per min and, if the concurrent NEFA R_a was assumed to be 3 × glycerol R_a = 7.86 μmol/kg per min and these NEFA were completely oxidized, the daily energy production from lipid oxidation would be 11.3 kJ/kg (based on 1 μmol of lipid = 13.9 kJ; Blaxter 1989). This, in turn, would account for approximately 20% of the estimated daily basal metabolic rate (BMR) of polar bears, the body masses of which ranged from 100 to 500 kg (based on BMR = 293·mass$^{0.75}$; Kleiber 1975). One explanation that could account for the low R_a values for glycerol in polar bears is that much of the glycerol released through lipolysis remains within adipocytes and is used to re-synthesize triacylglycerol (lipogenesis) via the activation of glycerol kinase. The presence and activity of glycerol kinase in polar bear adipose tissue has not been determined, but glycerol kinase activity has been observed to increase in humans and rats in association with obesity, consumption of high fat diets, and high plasma concentrations of glycerol or insulin (Persico et al. 1975, Stern et al. 1983, Chakrabarty et al. 1984), all of which are characteristic of polar bears. An enhanced activity of glycerol kinase in the adipose tissue of polar bears relative to that in other mammals also could function as a mechanism to regulate the supply of fatty acids to meet (and not exceed) the demand for fatty acid oxidation.

The rate of clearance (R_c) of plasma glycerol also was extremely slow in polar bears relative to values calculated for other species (for example, humans - R_c = 51.0±6.4 ml/kg per min, Beylot et al. 1987; dogs - R_c = 50.5±1.4 ml/kg per min, Previs et al. 1996; goats - R_c = 42.6±3.2 ml/kg per

min, Weber et al. 1993). Furthermore, the plasma glycerol concentration in polar bears was much higher than values reported for a wide range of other mammalian species (40-400 μmol/L reported in Lin 1977). These results indicate that the rate of tissue uptake of plasma glycerol and its loss by other routes, such as urine, were slow in polar bears relative to rates measured in other mammals. These results also are consistent with the finding of a previous study (Nelson et al. 1983) that the rate of disappearance of ^{14}C-glycerol from the plasma of free-ranging polar bears captured during summer was slower than rates observed for black bears both during summer activity and during winter dormancy (Ahlquist et al. 1984).

The R_a and R_c values for glycerol in polar bears were affected by differences in nutritional state (Table 4.2). Female polar bears captured during winter after emerging from their snow dens had significantly lower values than feeding bears, and fasting bears captured during summer-fall had values that were intermediate to those of feeding bears and fasting females captured during winter. Nevertheless, because it is unlikely that the glycerol R_a accurately reflected the rate of lipolysis, and because the fate of ^{13}C-glycerol in plasma was not determined, the significance of these differences is not known and requires further investigation.

Despite differences between feeding and fasting bears in plasma concentrations of acyl-carnitine (AC), free carnitine (FC), and total carnitine, the AC:FC ratio was not affected by nutritional state (Table 4.1 and Fig. 4.2). Typically, in a fasting mammal, carnitine biosynthesis and the release of carnitine stored in muscle are not sufficient to maintain a constant plasma concentration of FC in the face of the increased rate of carnitine acylation that occurs concomitantly with an increased rate of fatty acid oxidation (Hoppel and Genuth 1980). Thus, plasma AC values increase, plasma FC values decrease, and the AC:FC ratio rises as the rate of oxidation of NEFA rises. The fact that in polar bears the AC:FC ratio was not affected by nutritional state, and that the AC:FC ratio was low (< 1.0) in all animals, provides further support for the proposition that polar bears are able to balance precisely the synthesis, release, and use of lipid metabolites for energy metabolism whether the source is food or stored lipid in adipose tissue.

Fasting female polar bears captured during winter had significantly higher plasma

concentrations of AC and FC than did feeding or fasting bears captured during other seasons (Fig. 4.2). This indicates that these bears were well able to maintain a supply of carnitine through protein catabolism despite undergoing 8-9 months of fasting that was combined with pregnancy and 3-4 months of lactation. It is not clear why AC and FC values were two to three times higher in female polar bears captured after emerging from their winter dens than in polar bears captured during other seasons. It is possible that these higher values reflected an intense state of energy mobilization, as females captured after emerging from dens also have been found to have high plasma concentrations of glucagon, glucose, and cortisol, and a low insulin:glucagon molar ratio, when compared to bears captured during other seasons (see Chapter 6).

Plasma concentrations of AC and FC were higher in feeding males than in fasting males, but lower in feeding females than in fasting females (Fig. 4.2). The demand on body energy stores is greatest for females during summer-fall (in association with pregnancy and lactation) when food is less available or not available at all. Thus, protein catabolism should be greater in females during summer-fall than during spring, and their higher plasma AC and FC values may have reflected a concomitantly greater rate of carnitine biosynthesis (from lysine and methionine) and release (from muscle). Conversely, the demand on body energy stores is greatest for males during spring (in association with breeding activity) when, despite the availability of food, males will fast intermittently while seeking a mate. Thus, protein catabolism, and associated carnitine biosynthesis and release, should be greater for males during spring than during summer-fall. A similar interaction between sex and nutritional state affected the plasma concentration of total amino acids in polar bears (Chapter 3).

In mammals, cholesterol synthesis is reduced during fasting (Mayes 1993). The observation that plasma concentrations of total cholesterol, unesterified cholesterol, and HDL-cholesterol were lowest among female polar bears in fasting bears captured during summer-fall is consistent with this general statement (Table 4.1 and Fig. 4.3). The fact that female polar bears capturing during winter after emerging from their dens did not also have low cholesterol values, as would be expected given their fasting state, may have reflected an increased rate of metabolism in

these females relative to those captured during summer-fall. Metabolic rates for female polar bears captured during winter after emerging from snow dens have not been determined, but relative differences in rectal temperature and plasma thyroid hormone concentrations between fasting bears captured during summer-fall and fasting females captured during winter support the proposition that metabolic rates may be higher following den emergence (see Chapter 6).

The total cholesterol:HDL-cholesterol ratio ranged from 1.2 to 3.9 in the polar bears of this study (Fig. 4.3). These values are significantly lower than mean values measured in humans (females = 4.4, males = 5.0; Mayes 1993), but are similar to mean values determined for black bears (active = 1.6, denning = 2.7; Hasan 1997). The low ratios in polar and black bears indicate that HDL is the dominant lipoprotein in these species. Low density lipoprotein (LDL) is the predominant lipoprotein class in humans. In studies of comparative lipid and lipoprotein metabolism, species are classified as LDL versus HDL mammals (Bauer 1996). LDL mammals have a predominance of LDL-cholesterol which readily increases in concentration in response to high dietary fat and cholesterol consumption. In humans and some other species, these diet-induced increases in LDL-cholesterol are associated with atherogenesis, the formation of abnormal masses of lipid in arterial walls. By comparison, HDL mammals have a predominance of HDL which is thought to provide resistance to LDL-cholesterol elevations and associated atherogenesis.

Plasma triacylglycerol concentration was significantly affected by differences in nutritional state, but in a way that was dissimilar between sexes (Table 4.1). Female polar bears captured during winter after emerging from snow dens had lower values than females captured during other times of the year. Given that females appear to be in a state of intense energy mobilization following the emergence from their snow dens (see Chapter 6), low plasma levels of triacyglycerol may have reflected a low rate of hepatic lipogenesis. Fasting male bears had higher triacylglycerol values than did feeding male bears, but the reason for this is not known.

Differences among bears in plasma phospholipid concentration paralleled the differences among bears in HDL-cholesterol concentration (Table 4.1). This result is not surprising, given that the HDL-cholesterol of polar bears contains approximately 80% of the total phospholipid in plasma

(Kaduce et al. 1981).

4.4.2 Association with body condition and drug dosage

In this study, body condition was defined as the abundance of potential metabolic energy stored in an animal's tissues (primarily adipose tissue and skeletal muscle) relative to its body size. The body condition index (BCI) was developed to provide an estimate of the body condition of individual polar bears relative to other bears in a total sample population of 1072 animals (see Chapter 2). Although the BCI is an index without units, it can be used in conjunction with the body length of a bear to predict its mass of adipose tissue, skeletal muscle, or both. The range of BCI values is distributed normally around a mean value of zero, with positive values indicating a larger availability of potential metabolic energy and negative values indicating a smaller availability of potential metabolic energy, relative to the mean body condition of the sample population.

The BCI was negatively correlated with plasma concentrations of AC and FC, but was not correlated with the AC:FC ratio. This indicates that fat bears (BCI > 0) had lower AC and FC values than lean bears (BCI ≤ 0). The most likely explanation for this is that protein catabolism was slower in fat bears and, therefore, the biosynthesis and release of carnitine also was slower than in lean bears. Plasma concentrations of total cholesterol, unesterified cholesterol, and phospholipid also tended to be lower in fat bears than in lean bears.

The anesthetic drug Telazol® has not been demonstrated to have an effect on the plasma concentrations of lipids in a variety of mammals (Van Heerden et al. 1991, Lin et al. 1993, Peinado et al. 1993). The dosage of Telazol® administered to polar bears in this study did not correlate with either the plasma concentrations of lipids or the kinetic parameters of plasma glycerol. This suggests that the anesthetic protocol used with polar bears did not have a significant effect on the results of this study.

5. EFFECT OF DIET AND PROLONGED FASTING ON THE FATTY ACID COMPOSITION OF PLASMA AND ADIPOSE TISSUE IN POLAR AND BLACK BEARS

5.1 Introduction

Fatty acids are a heterogeneous group of compounds of biomedical importance. Aside from playing a prominent role in energy metabolism, fatty acids are required for somatic growth, reproduction, a responsive immune system, and the normal function of mitochondria, cell membranes, and skin. Their diversity of essential physiological roles is underscored by the many diseases linked to essential fatty acid (EFA) deficiency and imbalance. These include chronic malnutrition and anorexia nervosa (Holman et al. 1981, Holman et al. 1995), heart disease and atherosclerosis (Seidelin et al. 1992, Siscovick et al. 1995), depression and attention-deficit hyperactivity disorder (Hibbeln and Salem 1995, Stevens et al. 1995), childhood asthma and cystic fibrosis (Hodge et al. 1996, Lloyd-Still et al. 1996), and Reye's syndrome and Crohn's disease (Ogburn et al. 1982, Belluzzi et al. 1996).

Mammals have some metabolic capacity to alter or synthesize fatty acids through desaturase and elongase enzyme systems (Fig. 5.1). Nevertheless, normal fatty acid balance in tissues depends largely on diet because the dietary EFA's, linoleic (18:2ω6)[1] and α-linolenic (18:3ω3) acids, cannot be synthesized by mammals (Hadley 1985). Furthermore, because linoleic and α-linolenic acids are competitive substrates in the same metabolic cascade (Fig. 5.1), a diet must contain these EFA in correct proportions (Collier and Sinclair 1993).

[1] The "ωx" notation is used to indicate the position of double bonds in unsaturated fatty acids instead of the "*n-x*" notation recommended by the International Union of Pure and Applied Chemistry - International Union of Biochemistry (IUPAC-IUB Commission on Biochemical Nomenclature 1977). This nomenclature is used in recognition that the "ωx" notation was initiated in 1963 by Dr. Ralph T. Holman, a co-author on a version of this chapter which is to be submitted for publication.

Polar bears (*Ursus maritimus*) and black bears (*U. americanus*) have different diets but similar feeding strategies. Polar bears are marine carnivores that feed primarily on seals. Black bears are terrestrial omnivores, and consume largely vegetation. Both species fast annually for periods lasting 4-5 months in most cases, and as long as 8 months in the case of pregnant polar bears. In humans, fatty acid deficiency can develop rapidly during fasting or fat-free feeding (Imaichi et al. 1963, Paulsrud et al. 1972), as well as during pregnancy and lactation (Holman et al. 1991). Whether the proportions of EFA's are similar or differ between feeding and fasting bears has not been established.

In this study, the fatty acid composition of three lipid fractions (triacylglycerol, non-esterified fatty acid, and phospholipid) in plasma, and one lipid fraction in adipose tissue (triacylglycerol), was determined in polar and black bears during feeding and fasting. The objectives were to determine if the fatty acid composition of feeding polar bears and black bears was associated with the different diets, and to determine how the fatty acid composition of plasma and adipose tissue was associated with nutritional state (feeding versus fasting) within each species.

5.2 Materials and methods

One hundred and seven, free-ranging, adult polar bears were captured between July 1993 and November 1995. All bears were located from a helicopter and then anesthetized with Telazol® (8 to 10 mg/kg intramuscularly) using remote injection equipment (Stirling et al. 1989). Twenty-five adult polar bears were captured during spring (April and May) while they were hunting seals on arctic sea-ice near Resolute Bay, Northwest Territories, Canada (74°00' to 76°00'N and 93°00' to 100°50'W). Eighty-two adult polar bears were captured during summer, fall, and winter (July to March) while fasting on land along the west coast of Hudson Bay near Churchill, Manitoba, Canada (57°00' to 58°50'N and 92°25' to 94°15'W). Twenty milliliters of blood was collected from the jugular vein into Vacutainer® tubes containing sodium heparin (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). Blood samples were cooled immediately following collection and were centrifuged within 12 hours to collect and freeze plasma. Subcutaneous adipose tissue was

obtained by punch biopsy (6mm diameter), from the gluteal region of 13 feeding bears and 15 fasting bears, and was flash-frozen in liquid nitrogen. The frozen plasma and adipose tissue were shipped to the Hormel Institute, University of Minnesota, for determination of fatty acid composition using techniques previously described (Holman et al. 1994).

Thirty plasma and six adipose tissue samples were collected from 1989 to 1991 from three captive adult male black bears housed at the Carle Foundation Bear Research Facility in Urbana, Illinois. Food and water were provided *ad libitum*. Food was a complete commercial ration prepared with vegetable and terrestrial animal products and supplemented with vitamins and minerals. It contained a minimum of 22% crude protein, 9% crude fat, and 53% carbohydrate. The bears did not eat during winter dormancy (late November to mid-March) despite access to food and water. All bears were anesthetized with Telazol® (4 to 6 mg/kg intramuscularly) prior to collecting blood from their femoral vein and subcutaneous adipose tissue from their gluteal region. Ten blood samples were collected from each bear over a 3-year period, during different months, such that five samples were collected during feeding (late March to mid-November), and five during winter dormancy. Two adipose tissue samples were collected from each bear during 1991, such that one sample was collected during feeding and the other during winter dormancy. Blood plasma and adipose tissue samples were handled, processed, and analyzed as described for polar bears.

Chromatographic analyses detected fatty acids ranging from lauric (12:0) to lignoceric (24:0) acids, and included all saturated, unsaturated, branched-, and odd-chain fatty acids within this range (see Fig. 5.1). Fatty acid content was expressed as a percentage of total fatty acids within a lipid fraction.

Statistical analysis was performed using SPSS software (SPSS, Inc., Chicago, Illinois, USA). Student's *t*-test was used to test for significant differences in the proportions of individual fatty acids and groups of fatty acids and in the mean values of indices that are used to assess the proportions of essential fatty acids (EFA) in humans (see Table 5.1; Holman 1968, Holman et al. 1991), between species during feeding, and between feeding and fasting bears within each species (Zar 1996). The paired *t*-test was used to compare proportions of fatty acids in the adipose tissue

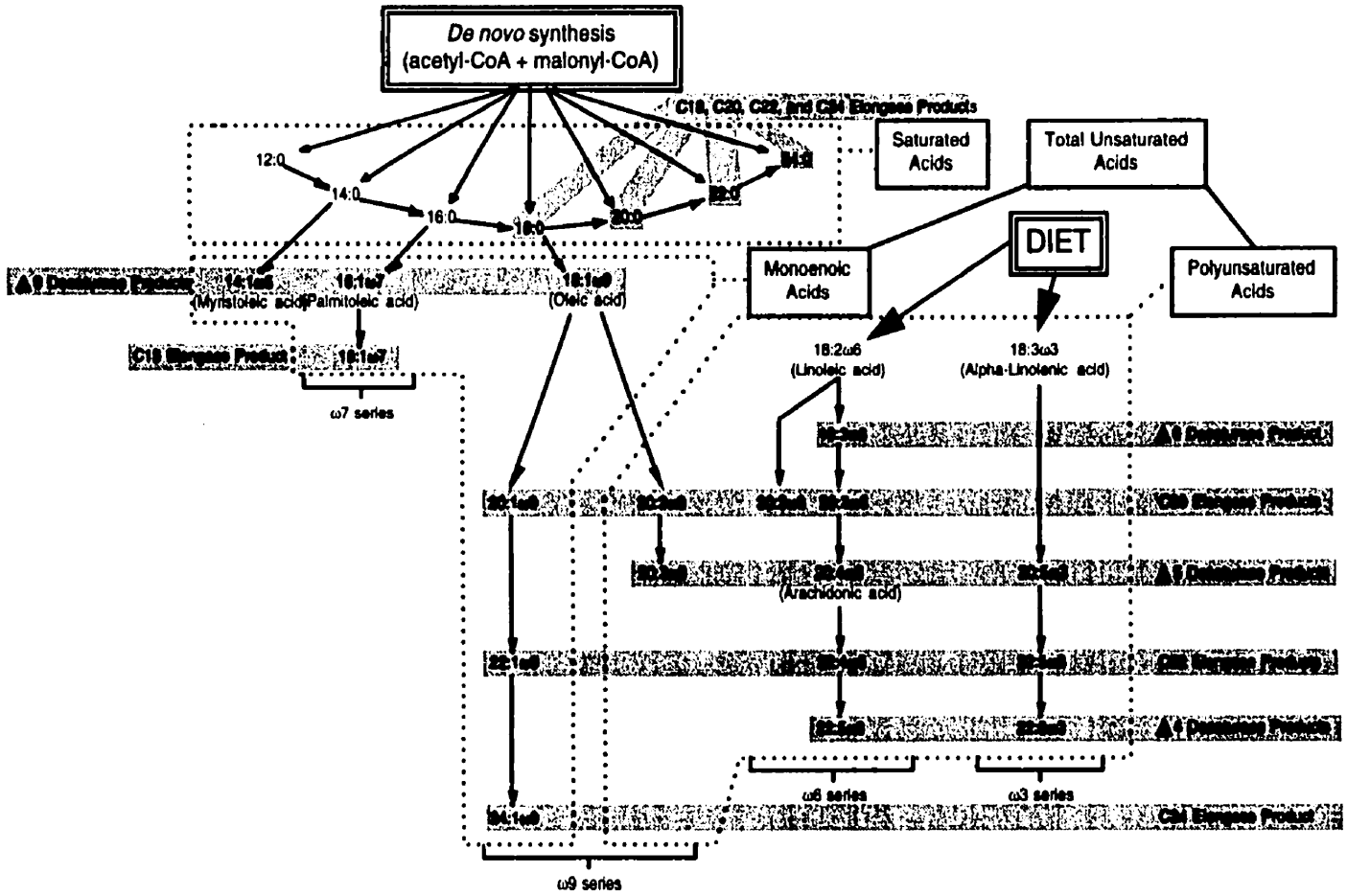


Figure 5.1 Classification and metabolic interrelationship of fatty acids detected in the plasma and adipose tissue of polar and black bears.

Table 5.1 Classification scheme used to describe fatty acids in the plasma of polar and black bears (see also Fig. 5.1).

Classification	Description
A. Structural Groupings	
ΣPUFA	- sum of polyunsaturated fatty acids
ΣMONO	- sum of monoenoic fatty acids
ΣSAT	- sum of saturated fatty acids
ΣBRANCH	- sum of branched chain fatty acids of 16:0, 17:0, and 18:0
ΣODD	- sum of odd-chain fatty acids, 15:0 and 17:0
B. Series Groupings	
Σω6	- sum of ω6 series
ω6 P	- sum of products of 18:2ω6 (linoleic acid)
Σω3	- sum of ω3 series
ω3 P	- sum of products of 18:3ω3 (α-linolenic acid)
Σω9	- sum of ω9 series
ω9 P	- sum of products of 18:1ω9 (oleic acid)
Σω7	- sum of ω7 series
ω7 P	- sum of product of 16:1ω7 (palmitoleic acid)
C. Desaturase Product Groupings	
Δ6 DP	- sum of product of Δ6 desaturase
Δ5 DP	- sum of products of Δ5 desaturase
Δ4 DP	- sum of products of Δ4 desaturase
Δ9 DP	- sum of products of Δ9 desaturase
D. Elongase Product Groupings	
C18 EP	- sum of product of 18 carbon elongase
C20 EP	- sum of products of 20 carbon elongase
C22 EP	- sum of products of 22 carbon elongase
C24 EP	- sum of product of 24 carbon elongase
E. Indices	
TRI:TET ratio	- ratio of the triene, 20:3ω9, to the tetraene, 20:4ω6
E:NE ratio	- ratio of dietary essential fatty acids and products (Σω6 + Σω3) to non-essential fatty acids and products
ω6: ω3 ratio	- ratio of Σω6 to Σω3
MCL	- mean chain length in carbon atoms, calculated as the average number of carbon atoms per fatty acid within a lipid fraction
DBI	- double bond index, calculated as the average number of double bonds per fatty acid within a lipid fraction

collected from black bears during feeding and winter dormancy. Because of the high degree of interdependency between variables (as a result of being expressed as a percentage) and because of the large number of pairwise comparisons, the Dunn-Šidák method (Ury 1976) was used to calculate a more conservative value for statistical significance, $p \leq 0.017$, rather than the commonly used value of $p \leq 0.05$.

5.3 Results

5.3.1 Plasma fatty acid composition in feeding polar and black bears

5.3.1.1 *Triacylglycerol*

The proportions and indices of fatty acids and fatty acid groupings in triacylglycerol differed markedly between species (Table 5.2 and Fig. 5.2). Despite a relative abundance of ω3 fatty acids in polar bears, and ω6 fatty acids in black bears, there were no statistically significant differences between species in the ratio of the sum of dietary EFA's and products to the sum of non-essential fatty acids and products (E:NE ratio), or in the sum of polyunsaturated fatty acids (ΣPUFA). On average, the triacylglycerol fatty acids of polar bears were longer and more unsaturated than those of black bears.

5.3.1.2 *Non-esterified fatty acid*

The proportions and indices of fatty acids and fatty acid groupings in non-esterified fatty acid differed markedly between species, and many of these differences were similar to those in the triacylglycerol fraction (Table 5.3 and Fig. 5.2). Some exceptions, however, were that the proportion of 20:4ω6 was greater in black bears than in polar bears, and the proportion of 18:1ω9 and the ratio of the proportion of the triene 20:3ω9 to the proportion of the tetraene 20:4ω6 (TRI:TET) were similar in both species.

Table 5.2 Proportions and indices of fatty acids in the plasma triacylglycerol of polar and black bears during feeding and fasting.[a]

Classification	Polar Bear (A) Feeding ($n = 25$)	Polar Bear (B) Fasting ($n = 82$)	Black Bear (C) Feeding ($n = 15$)	Black Bear (D) Fasting ($n = 15$)	Comparison[b] A vs C	A vs B	C vs D
A. Fatty Acid							
18:2ω6	2.81±0.17	6.64±0.15	26.71±0.59	23.74±0.36	***	***	***
18:3ω6	0.20±0.01	0.17±0.01	0.99±0.10	0.44±0.03	***	ns	***
20:2ω6	0.33±0.03	0.17±0.02	0.86±0.07	0.35±0.01	***	***	***
20:3ω6	0.20±0.02	0.25±0.02	1.05±0.08	0.80±0.05	***	**	***
20:4ω6	2.35±0.15	1.99±0.05	2.55±0.16	3.84±0.15	ns	***	***
22:4ω6	0.28±0.05	0.19±0.02	1.10±0.11	0.71±0.03	***	**	***
22:5ω6	0.07±0.01	0.15±0.01	0.65±0.08	0.25±0.02	***	***	***
18:3ω3	0.48±0.02	0.93±0.04	1.02±0.05	0.64±0.03	***	***	***
20:5ω3	13.74±1.48	6.59±0.28	0.17±0.03	0.10±0.02	***	***	ns
22:5ω3	4.32±0.09	3.42±0.09	0.53±0.05	0.64±0.02	***	***	***
22:6ω3	15.18±0.64	17.06±0.43	0.24±0.03	0.40±0.05	***	***	**
20:2ω9	nd	0.07±0.02	nd	nd	ns	***	ns
20:3ω9	0.01±0.01	0.09±0.01	1.08±0.09	0.64±0.02	***	***	***
18:1ω9	22.26±0.98	20.41±0.32	31.33±0.35	24.52±0.26	***	***	***
20:1ω9	2.30±0.26	1.07±0.06	0.62±0.06	0.29±0.02	***	***	***
22:1ω9	nd	0.01±0.01	0.05± 0.02	0.02±0.01	***	**	**
24:1ω9	nd	nd	nd	nd	ns	ns	ns
16:1ω7	13.72±0.63	11.74±0.24	4.46±0.21	4.40±0.36	***	***	ns
18:1ω7	6.25±0.30	6.99±0.21	4.35±0.13	6.50±0.33	***	**	***
14:1ω5	0.64±0.04	0.72±0.02	0.14±0.02	0.14±0.02	***	**	ns
12:0	4.98±0.53	7.02±0.29	0.01± 0.004	0.02 ± 0.004	***	***	ns
14:0	1.45±0.14	1.38±0.04	0.73±0.02	0.72±0.05	***	ns	ns
16:0	5.57±0.52	8.09±0.33	15.85±0.53	26.39±0.38	***	***	***
18:0	2.61±0.26	4.21±0.23	3.40±0.28	2.55±0.19	**	***	***
20:0	nd	0.06±0.01	0.05±0.01	0.02±0.01	***	***	***
22:0	0.12±0.03	0.04±0.01	0.14±0.03	0.09±0.02	ns	***	**
24:0	nd	nd	0.03±0.03	nd	***	ns	***
ΣBRANCH	nd	0.35±0.06	1.40±0.10	1.26±0.11	***	***	ns
ΣODD	0.15±0.01	0.37±0.04	1.04±0.07	0.93±0.06	***	***	ns
B. Index							
TRI:TET ratio	0.001± 0.0004	0.05±0.01	0.46±0.06	0.17± 0.01	***	***	***
E:NE ratio	0.70±0.05	0.61±0.01	0.56±0.02	0.47±0.01	ns	ns	***
ω6: ω3 ratio	0.19±0.01	0.36±0.01	17.52±0.56	17.36±0.74	***	***	ns
MCL	18.40±0.06	18.13±0.03	17.74±0.02	17.50± 0.01	***	***	***
DBI	2.46±0.08	2.21±0.03	1.31±0.02	1.17±0.01	***	**	***

[a] Proportions expressed as mean percentage of total fatty acids in triacylglycerol ± standard error of mean. Fatty acids that occurred at levels too low to be detected are indicated by "nd" for not detected.

[b] Student's *t*-test was used to compare between groups. Significance was assigned at $p \leq 0.017$ based on the Dunn-Šidák method (Ury 1976) and is indicated by "ns" for non-significance, ** for $p \leq 0.017$, and *** for $p \leq 0.001$.

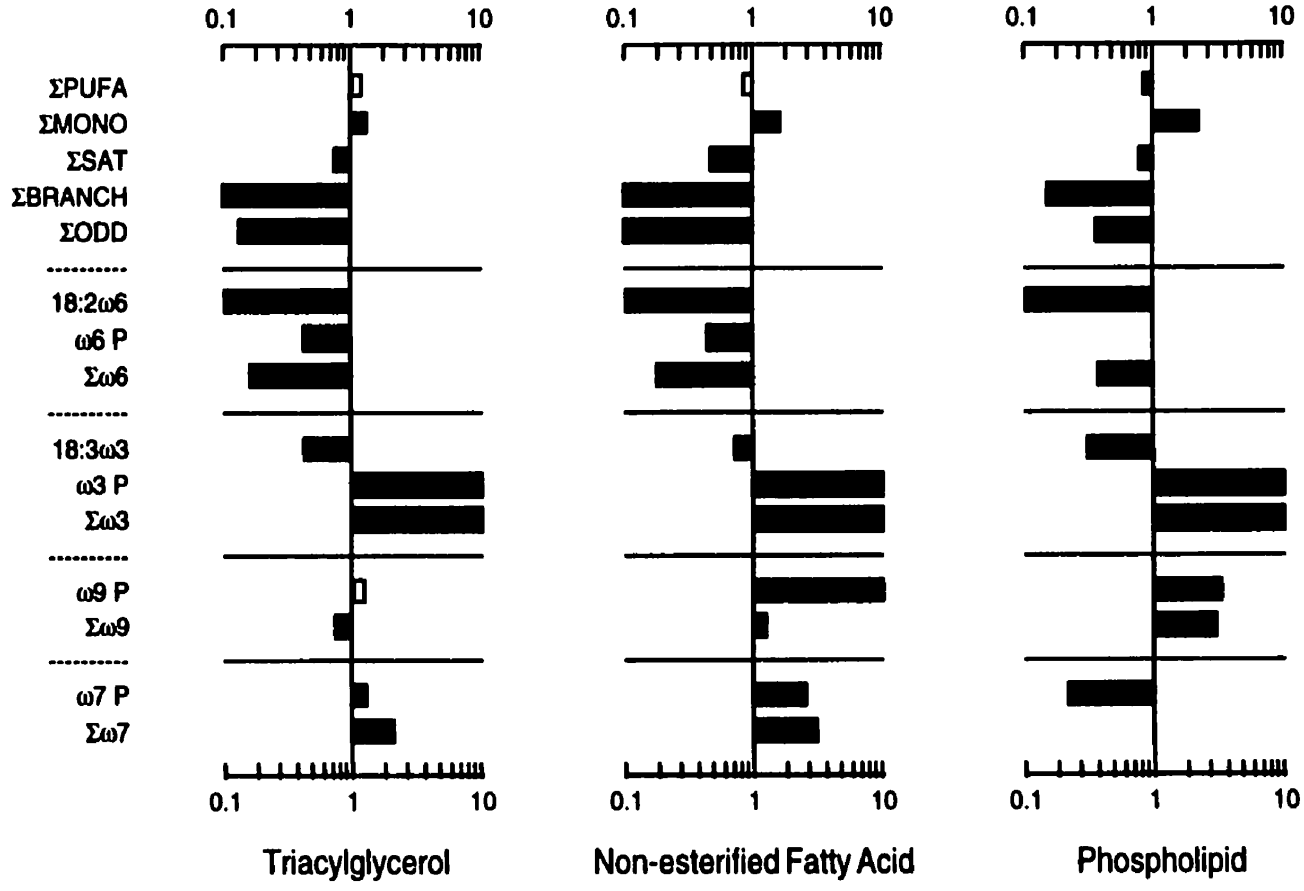


Figure 5.2 Comparison of dietary essential fatty acids and fatty acid groups between feeding polar and black bears. Ratios of mean values (i.e., [mean value for polar bear] ÷ [mean value for black bear]) are indicated on a logarithmic scale by bars for individual fatty acids and groups of fatty acids. The direction and length of the bars indicate the value of the ratio in relation to a value of 1, as well as the magnitude of difference between the groups being compared. The shading of the bars indicates the degree of significance: open bars are non-significant, cross-hatched bars are $p \leq 0.01$, and black bars are $p \leq 0.001$ for the comparison of ratios.

Table 5.3 Proportions and indices of fatty acids in the plasma non-esterified fatty acid of polar and black bears during feeding and fasting.[a]

Classification	Polar Bear (A) Feeding (n = 25)	Polar Bear (B) Fasting (n = 82)	Black Bear (C) Feeding (n = 15)	Black Bear (D) Fasting (n = 15)	Comparison[b] A vs C	A vs B	C vs D
A. Fatty Acid							
18:2ω6	1.80±0.09	3.80±0.15	20.53±1.34	15.93±0.44	***	***	***
18:3ω6	0.18±0.01	0.31±0.02	0.28±0.03	0.13±0.01	***	***	***
20:2ω6	0.19±0.01	0.33±0.02	0.36±0.04	0.25±0.03	***	***	ns
20:3ω6	0.27±0.03	0.46±0.05	1.02±0.10	0.42±0.05	***	***	***
20:4ω6	3.20±0.42	3.17±0.25	6.47±0.81	4.46±0.71	***	ns	**
22:4ω6	0.04±0.01	0.06±0.01	0.32±0.04	0.22±0.04	***	ns	**
22:5ω6	0.25±0.21	0.10±0.01	0.19±0.03	0.09±0.02	ns	***	***
18:3ω3	0.40±0.03	0.78±0.03	0.57±0.04	0.71±0.05	***	***	**
20:5ω3	9.29±1.00	6.97±0.34	0.34±0.06	0.09±0.02	***	***	***
22:5ω3	4.48±0.18	4.24±0.10	0.19±0.04	0.13±0.03	***	ns	ns
22:6ω3	6.63±0.45	10.21±0.20	0.54±0.06	0.53±0.07	***	***	ns
20:2ω9	nd	0.28±0.05	nd	nd	ns	***	ns
20:3ω9	0.05±0.01	0.07±0.01	0.14±0.03	0.11±0.02	***	***	ns
18:1ω9	22.66±0.79	21.53±0.40	24.34±1.04	29.88±1.08	ns	**	***
20:1ω9	8.46±0.67	4.67±0.19	0.27±0.02	0.26±0.02	***	***	ns
22:1ω9	0.79±0.09	0.15±0.02	nd	nd	***	***	***
24:1ω9	0.01±0.01	nd	nd	nd	ns	***	***
16:1ω7	16.64±0.86	16.04±0.42	4.50±0.40	6.60±0.42	***	ns	***
18:1ω7	6.96±0.28	8.03±0.16	2.43±0.19	3.29±0.24	***	***	**
14:1ω5	0.53±0.06	0.73±0.02	0.49±0.04	0.71±0.04	ns	***	***
12:0	0.04±0.01	0.45±0.08	0.40±0.05	0.17±0.04	***	***	***
14:0	2.17±0.16	2.13±0.06	1.56±0.24	1.22±0.13	**	ns	**
16:0	8.60±0.35	9.37±0.13	21.50±0.93	24.38±0.80	***	***	**
18:0	4.93±0.34	4.75±0.17	7.56±0.33	5.74±0.40	***	ns	**
20:0	nd	0.18±0.04	0.48±0.08	0.21±0.04	***	***	***
22:0	1.18±0.27	0.46±0.04	1.36±0.21	0.64±0.15	ns	***	***
24:0	nd	0.02±0.01	0.29±0.03	0.23±0.02	***	***	ns
ΣBRANCH	nd	0.38±0.07	3.22±0.23	3.07±0.22	***	***	ns
ΣODD	0.23±0.01	0.34±0.02	2.45±0.20	2.28±0.21	***	***	ns
B. Index							
TRI:TET ratio	0.02±0.004	0.03±0.004	0.04±0.02	0.04±0.01	ns	ns	ns
E:NE ratio	0.38±0.03	0.44±0.01	0.47±0.05	0.30±0.02	ns	ns	**
ω6: ω3 ratio	0.30±0.02	0.38±0.02	18.11±0.76	15.10±0.72	***	**	**
MCL	18.34±0.05	18.26±0.02	17.62±0.05	17.44±0.04	***	ns	**
DBI	1.86±0.06	1.96±0.02	1.14±0.05	1.01±0.03	***	ns	ns

[a] Proportions expressed as mean percentage of total fatty acids in non-esterified fatty acid ± standard error of mean. Fatty acids that occurred at levels too low to be detected are indicated by "nd" for not detected.

[b] Student's t-test was used to compare between groups. Significance was assigned at $p \leq 0.017$ based on the Dunn-Šidák method (Ury 1976) and is indicated by "ns" for non-significance, ** for $p \leq 0.017$, and *** for $p \leq 0.001$.

5.3.1.3 *Phospholipid*

As in the other lipid fractions, the proportions and indices of fatty acids and fatty acid groupings in phospholipid differed markedly between species (Table 5.4 and Fig. 5.2). However, there also were species differences, as well as features of the fatty acid composition of phospholipid in both species, that contrasted with those in the triacylglycerol and non-esterified fatty acid fractions. The proportion of 20:4ω6 was higher in phospholipid than in the other fractions and, when comparing species, the proportion of 20:4ω6 was greater in polar bears. Fatty acids of the ω7 series (Σω7) and 14:1ω5 were less prominent in phospholipid than in other fractions, especially in polar bears, whereas 18:0 was more prominent and the ratio of the sum of the ω6 series to the sum of the ω3 series (ω6:ω3 ratio) was higher in both species.

5.3.2 Plasma and adipose tissue fatty acid composition in feeding and fasting polar bears

5.3.2.1 *Plasma lipid fractions*

Differences in the proportions and indices of fatty acids and fatty acid groupings in the plasma lipid fractions between feeding and fasting polar bears were generally of lesser magnitude than many of the differences between species described above (Tables 5.2 to 5.4 and Fig. 5.3). The E:NE ratio did not differ significantly between feeding and fasting bears in any of the lipid fractions. The proportions of 18:2ω6 and 18:3ω3 were greater in the lipid fractions of fasting bears than in those of feeding bears. The ω6:ω3 ratio also was greater in fasting bears, largely as a result of corresponding inverse differences in the proportions of 18:2ω6 and 20:5ω3. The proportion of 20:4ω6 remained similar in both non-esterified fatty acid and phospholipid fractions between feeding and fasting bears, and was slightly lower in the triacylglycerol fraction of fasting bears.

5.3.2.2 *Plasma triacylglycerol*

Fatty acids in the triacylglycerol fraction of fasting polar bears generally were shorter, less saturated, and of greater diversity than in feeding polar bears (Table 5.2 and Fig. 5.3). The ratio of 20:3ω9 to 20:4ω9 (TRI:TET ratio) was greater in fasting bears, but well within the range indicative

Table 5.4 Proportions and indices of fatty acids in the plasma phospholipid of polar and black bears during feeding and fasting.[a]

Classification	Polar Bear		Black Bear		Comparison[b]		
	(A) Feeding (n = 25)	(B) Fasting (n = 82)	(C) Feeding (n = 15)	(D) Fasting (n = 15)	A vs C	A vs B	C vs D
A. Fatty Acid							
18:2ω6	2.34±0.13	6.09±0.23	26.51±0.91	17.48±0.77	***	***	***
18:3ω6	0.23±0.01	0.18±0.01	0.23±0.01	0.12±0.01	ns	***	***
20:2ω6	0.38±0.02	0.30±0.01	0.63±0.04	0.25±0.03	***	***	***
20:3ω6	1.60±0.10	2.10±0.08	2.68±0.13	2.83±0.25	***	***	ns
20:4ω6	12.65±0.64	12.66±0.27	10.66±0.78	16.35±1.00	**	ns	***
22:4ω6	0.15±0.01	0.14± 0.004	0.67±0.02	0.72±0.05	***	ns	ns
22:5ω6	0.07±0.01	0.19±0.01	0.26±0.02	0.26±0.02	***	***	ns
18:3ω3	0.05±0.01	0.10±0.01	0.14±0.01	0.07±0.01	***	**	***
20:5ω3	13.65±1.11	6.82±0.28	0.23±0.01	0.10±0.01	***	***	***
22:5ω3	2.13±0.06	1.66±0.03	0.49±0.03	0.85±0.04	***	***	***
22:6ω3	3.01±0.09	4.63±0.16	0.70±0.06	1.61±0.11	***	***	***
20:2ω9	nd	nd	nd	nd	ns	ns	ns
20:3ω9	0.18±0.02	0.21±0.01	0.28±0.02	0.27±0.02	***	ns	ns
18:1ω9	20.09±0.46	15.08±0.60	6.23±0.32	4.62±0.29	***	***	***
20:1ω9	2.10±0.25	0.81±0.04	0.27±0.02	0.14±0.01	***	***	***
22:1ω9	0.17±0.01	0.13±0.01	0.01±0.004	0.02±0.01	***	***	***
24:1ω9	2.05±0.17	2.24±0.06	0.68±0.06	0.80±0.06	***	**	ns
16:1ω7	3.44±0.19	2.69±0.06	0.80±0.04	0.49±0.02	***	***	***
18:1ω7	0.85±0.13	3.19±0.42	3.38±0.23	3.55±0.21	***	***	ns
14:1ω5	0.01±0.004	0.07±0.01	nd	nd	ns	***	ns
12:0	0.01±0.004	0.09±0.02	nd	nd	ns	***	ns
14:0	0.62±0.03	0.53±0.01	0.20±0.01	0.18±0.02	***	***	ns
16:0	11.42±0.26	11.28±0.15	15.76±0.44	22.33±0.63	***	ns	***
18:0	21.44±0.95	26.69±0.43	26.08±0.84	23.92±1.15	***	***	ns
20:0	0.81±0.06	0.95±0.03	0.37±0.02	0.37±0.03	***	***	ns
22:0	0.17±0.01	0.28±0.01	0.28±0.03	0.25±0.03	***	***	ns
24:0	0.01±0.01	0.12±0.04	0.29±0.03	0.20±0.02	***	***	**
ΣBRANCH	0.24±0.08	0.56±0.08	1.46±0.12	1.66±0.09	***	***	ns
ΣODD	0.25±0.02	0.44±0.04	0.63±0.04	0.80±0.04	***	***	**
B. Index							
TRI:TET ratio	0.01±0.004	0.02±0.004	0.03±0.004	0.02±0.004	***	ns	**
E:NE ratio	0.58±0.03	0.54±0.01	0.78±0.03	0.70±0.04	***	ns	ns
ω6: ω3 ratio	1.00±0.07	1.74±0.07	27.84±1.69	14.73±0.60	***	***	***
MCL	18.65±0.02	18.58±0.01	18.00±0.03	18.06±0.04	***	**	ns
DBI	1.89±0.06	1.67±0.01	1.30±0.03	1.39±0.05	***	***	ns

[a] Proportions expressed as mean percentage of total fatty acids in phospholipid ± standard error of mean. Fatty acids that occurred at levels too low to be detected are indicated by "nd" for not detected.

[b] Student's t-test was used to compare between groups. Significance was assigned at $p \leq 0.017$ based on the Dunn-Šidák method (Ury 1976) and is indicated by "ns" for non-significance, ** for $p \leq 0.017$, and *** for $p \leq 0.001$.

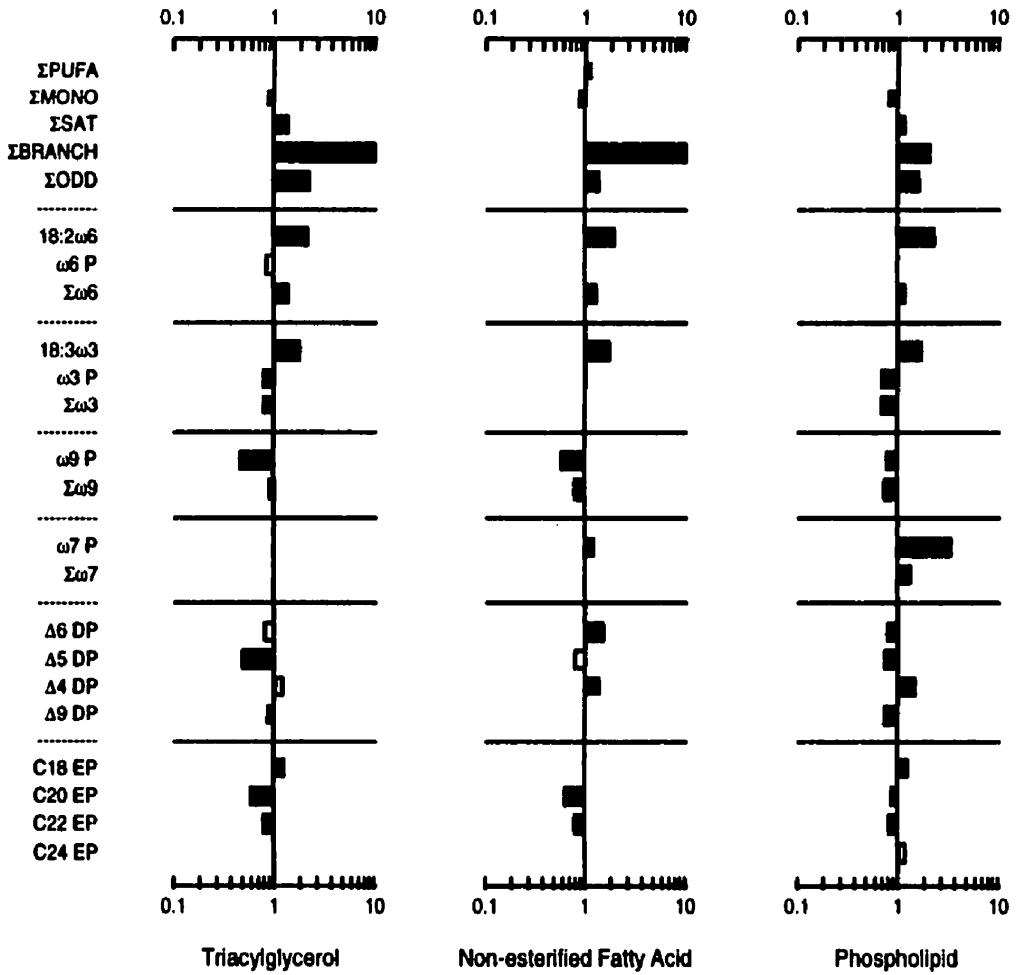


Figure 5.3 Comparison of dietary essential fatty acids, fatty acid groups, and desaturase and elongase products between fasting and feeding polar bears. Ratios are calculated as [mean value for fasting polar bear] ÷ [mean value for feeding polar bear]. Additional information in regard to the interpretation of the figure is provided in Figure 5.2.

of normal EFA status, i.e., < 0.4 (Holman 1968). Fasting female bears accompanied by new-born cubs had a significantly higher proportion of 20:4ω6 than other fasting adult bears, but the essential fatty acid indices (TRI:TET, E:NE, and ω6:ω3 ratios) were similar among groups (Table 5.6).

5.3.2.3 *Plasma non-esterified fatty acid*

In contrast to the triacylglycerol fraction, there were no differences in the TRI:TET ratio, the average carbon chain length of fatty acids (MCL), or the average number of double bonds per fatty acid (DBI) in the non-esterified fatty acid fraction between feeding and fasting polar bears (Table 5.3 and Fig. 5.3). However, fasting bears had a significantly higher proportion of the Δ4 desaturase product 22:6ω3 than did feeding bears. Fasting female bears accompanied by new-born cubs had a significantly lower proportion of 18:3ω3 than other fasting adult bears, but the essential fatty acid indices were similar among groups (Table 5.6).

5.3.2.4 *Plasma phospholipid*

Fasting polar bears had shorter and more saturated fatty acids in the phospholipid fraction than did feeding polar bears (Table 5.4 and Fig. 5.3). The TRI:TET ratio was similar between feeding and fasting bears. Fasting bears had higher proportions of Δ4 desaturase products (Δ4 DP) than did feeding bears. Fasting female bears accompanied by new-born cubs had a significantly greater proportion of 18:2ω6 than other fasting adult bears, but the essential fatty acid indices were similar among groups (Table 5.6).

5.3.2.5 *Adipose tissue triacylglycerol*

There were few differences in the fatty acid composition of adipose tissue triacylglycerol between feeding and fasting polar bears (Table 5.5 and Fig. 5.5). Fasting bears had slightly greater proportions of 18:2ω6 and 18:3ω3 than did feeding bears. Fasting bears also had lower proportions of the Δ5 desaturase products, 20:4ω6 and 20:5ω3, and a lower E:NE ratio, than did feeding bears.

Table 5.5 Proportions and indices of fatty acids in the adipose triacylglycerol of polar and black bears during feeding and fasting.[a]

Classification	Polar Bear (A) Feeding (n = 13)	Polar Bear (B) Fasting (n = 15)	Black Bear (C) Feeding (n = 3)	Black Bear (D) Fasting (n = 3)	Comparison[b] A vs B	Comparison[b] C vs D
A. Fatty Acid						
18:2ω6	2.65±0.04	2.82±0.06	14.77±0.41	9.37±0.17	*	**
18:3ω6	0.14±0.01	0.13±0.01	0.07±0.003	0.08±0.02	ns	ns
20:2ω6	0.18±0.02	0.23±0.02	0.29±0.003	0.21±0.02	*	*
20:3ω6	0.13±0.01	0.13±0.01	0.14±0.003	0.07±0.02	ns	*
20:4ω6	0.38±0.02	0.31±0.02	0.15±0.02	0.06±0.02	*	*
22:4ω6	0.12±0.01	0.09±0.02	nd	nd	ns	ns
22:5ω6	0.10±0.01	0.08±0.01	nd	nd	ns	ns
18:3ω3	0.46±0.02	0.50±0.04	0.61±0.06	0.42±0.02	ns	*
20:5ω3	4.43±0.27	2.99±0.27	nd	nd	***	ns
22:5ω3	6.66±0.18	6.47±0.19	nd	nd	ns	ns
22:6ω3	9.82±0.34	8.52±0.37	nd	nd	*	ns
20:2ω9	nd	nd	nd	nd	ns	ns
20:3ω9	0.04±0.004	0.04±0.01	nd	nd	ns	ns
18:1ω9	24.34±0.43	26.76±0.84	47.61±0.84	46.10±0.74	*	ns
20:1ω9	5.62±0.36	6.69±0.51	1.00±0.02	0.63±0.06	ns	*
22:1ω9	0.17±0.01	0.18±0.02	nd	nd	ns	ns
24:1ω9	nd	nd	nd	nd	ns	ns
16:1ω7	22.18±0.32	21.80±0.82	10.15±1.49	8.39±0.34	ns	ns
18:1ω7	6.24±0.22	6.30±0.12	0.74±0.18	0.32±0.05	ns	ns
14:1ω5	1.74±0.04	1.55±0.06	0.49±0.04	0.37±0.04	*	ns
12:0	nd	nd	nd	nd	ns	ns
14:0	4.11±0.08	3.85±0.08	2.07±0.12	1.67±0.13	*	*
16:0	8.00±0.18	8.00±0.26	20.42±1.05	27.69±2.28	ns	*
18:0	1.84±0.10	1.96±0.11	0.61±0.05	4.01±0.39	ns	**
20:0	nd	nd	0.24±0.03	0.13±0.01	ns	*
22:0	0.31±0.03	0.30±0.04	nd	nd	ns	ns
24:0	nd	nd	nd	nd	ns	ns
ΣBRANCH	nd	nd	nd	nd	ns	ns
ΣODD	0.34±0.01	0.31±0.01	0.62±0.27	0.47±0.19	ns	ns
B. Index						
TRI:TET ratio	0.12±0.01	0.13±0.03	0.00	0.00	ns	ns
E:NE ratio	0.34±0.01	0.29±0.01	0.19±0.01	0.11±0.002	*	**
ω6: ω3 ratio	0.18±0.01	0.21±0.01	25.62±2.43	23.24±1.22	*	ns
MCL	18.05±0.03	18.01±0.04	17.30±0.02	17.20±0.07	ns	ns
DBI	1.85±0.03	1.72±0.04	0.93±0.02	0.77±0.03	*	*

[a] Proportions expressed as mean percentage of total fatty acids in triacylglycerol ± standard error of mean. Fatty acids that occurred at levels too low to be detected are indicated by "nd" for not detected.

[b] Student's t-test was used to compare between feeding and fasting polar bears, whereas the paired t-test was used to compare between feeding and fasting black bears. Significance was assigned at $p \leq 0.05$ and is indicated by "ns" for non-significance, * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

5.3.3 Plasma and adipose tissue fatty acid composition in feeding and fasting black bears

Differences in the proportions and indices of fatty acids and fatty acid groupings in the different lipid fractions between feeding and fasting black bears were generally of lesser magnitude than many of the differences between species described above (Tables 5.2 to 5.4 and Fig. 5.4). Fasting black bears had a lower proportion of 18:2ω6 in all lipid fractions, and a lower proportion of 18:3ω3 in triacylglycerol and phospholipid fractions, than did feeding black bears.

5.3.3.1 *Plasma triacylglycerol*

Fatty acids in the triacylglycerol fraction of fasting black bears were generally shorter and less saturated than in feeding black bears (Table 5.2 and Fig. 5.4). The proportions of the diet-derived EFA, 18:2ω6 and 18:3ω3, were significantly lower in fasting bears, as were the TRI:TET and E:NE ratios. However, the proportions of 20:4ω6 and the ω3 products (ω3 P), 22:5ω3 and 22:6ω3, were greater in fasting bears than in feeding bears.

5.3.3.2 *Plasma non-esterified fatty acid*

The differences in the proportions and indices of fatty acids and fatty acid groupings in the non-esterified lipid fraction between feeding and fasting black bears contrasted in some ways with the differences that occurred in the triacylglycerol fraction (Table 5.3 and Fig. 5.4). The E:NE ratio, ΣPUFA, and proportions of 18:2ω6 and 20:4ω6 were lower in fasting bears than in feeding bears, whereas the TRI:TET ratio did not differ between the two groups and the proportion of 18:3ω3 was greater in the fasting bears.

5.3.3.3 *Plasma phospholipid*

The differences in the proportions and indices of fatty acids and fatty acid groupings in the phospholipid fraction between feeding and fasting black bears were similar to the differences that occurred in the triacylglycerol fraction (Table 5.4 and Fig. 5.4). The proportions of 18:2ω6 and 18:3ω3 and the TRI:TET ratio were lower in fasting bears than in feeding bears, but the E:NE ratio

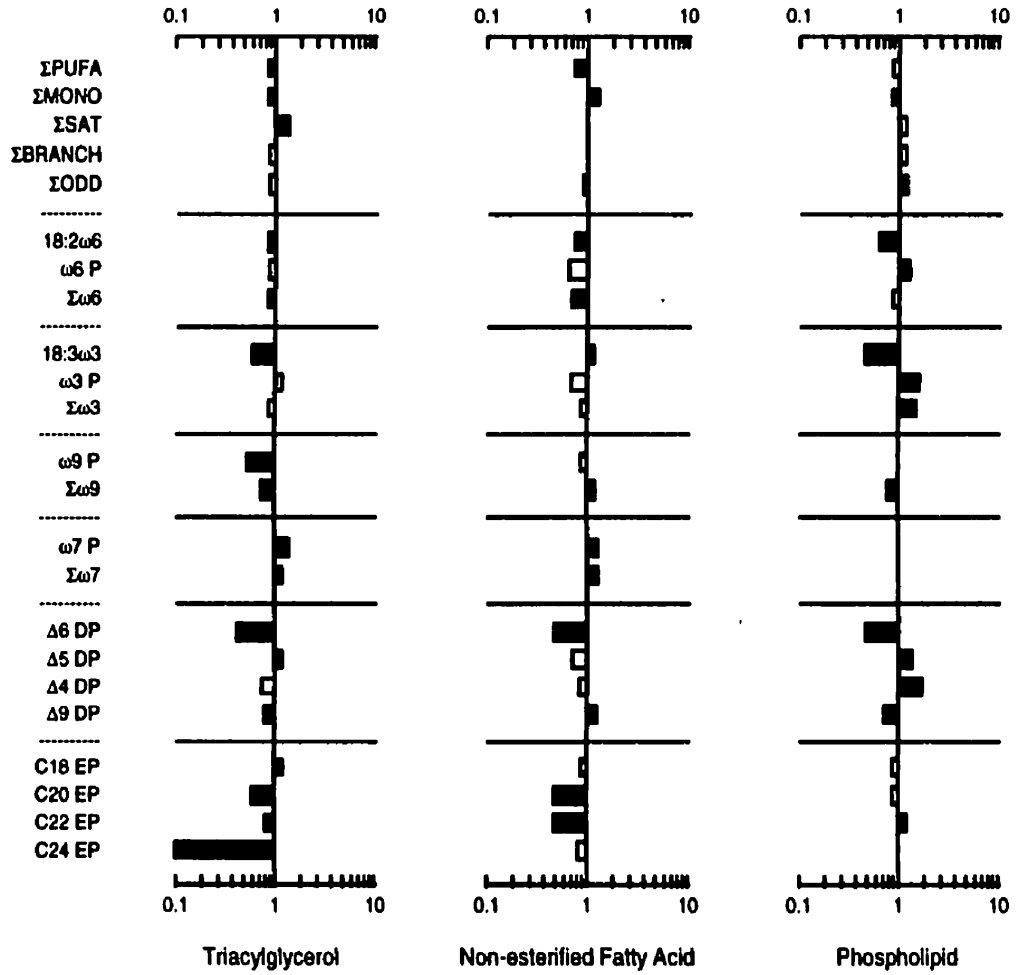


Figure 5.4 Comparison of dietary essential fatty acids, fatty acid groups, and desaturase and elongase products between fasting and feeding black bears. Ratios are calculated as [mean value for fasting black bear] ÷ [mean value for feeding black bear]. Additional information in regard to the interpretation of the figure is provided in Figure 5.2.

and ΣPUFA did not differ between groups. Fasting bears had greater proportions of 20:4ω6 and the ω3 products, 22:5ω3 and 22:6ω3, than did feeding bears.

5.3.3.4 *Adipose tissue triacylglycerol*

There were numerous differences in the fatty acid composition of adipose tissue triacylglycerol between feeding and fasting black bears (Table 5.5 and Fig. 5.5). Fasting bears had significantly lower proportions of 18:2ω6, 20:4ω6, 18:3ω3, and ω3 products (ω3 P) than did feeding bears. Consequently, the E:NE ratio was significantly lower in fasting bears.

5.4 Discussion

5.4.1 Plasma fatty acid composition in feeding polar and black bears

No published data exist on the fatty acid composition of plasma in free-ranging black bears. The captive black bears sampled in this study were not consuming a natural diet but consumed food of terrestrial origin as do free-ranging black bears under natural conditions. The captive bears also underwent a 4-month period of winter dormancy each year, as do free-ranging black bears. Thus, the basic comparisons between species during feeding, and between feeding and fasting black bears, are broadly legitimate.

The fatty acid composition of plasma lipid fractions differed markedly between feeding polar and black bears, and appeared to be strongly influenced by diet. Of the three lipid fractions considered, triacylglycerol was probably most affected by diet because much of the triacylglycerol in plasma is absorbed directly from food (Ganong 1995). The plasma triacylglycerol of polar bears contained a large proportion of ω3 fatty acids (Table 5.2 and Fig. 5.2), especially eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids, as has been reported previously (Kaduce et al. 1981, Innis and Kuhnlein 1987). These acids are synthesized by marine algae and phytoplankton and are transferred through the food chain to fish and marine mammals, and ultimately to carnivorous mammals which consume them. In contrast, the plasma triacylglycerol of black bears contained a

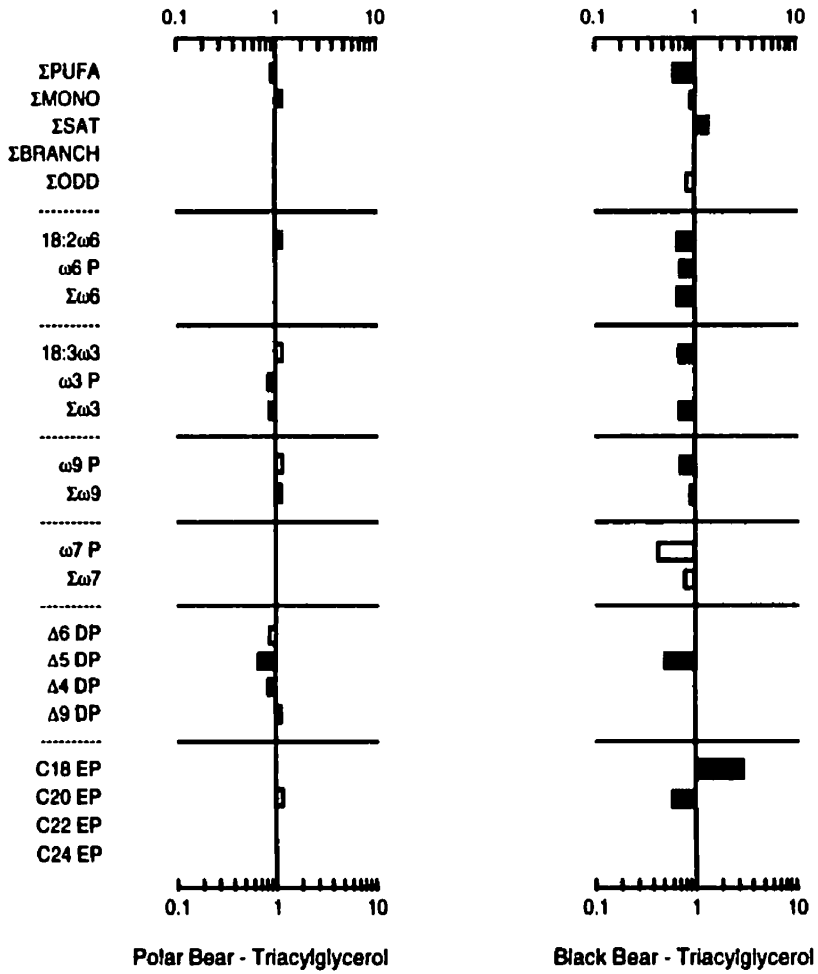


Figure 5.5 Comparison of dietary essential fatty acids, fatty acid groups, and desaturase and elongase products between fasting and feeding in the adipose tissue triacylglycerol of polar and black bears. Ratios are calculated as: [mean value for fasting bear] ÷ [mean value for feeding bear]. Additional information in regard to the interpretation of the figure is provided in Figure 5.2.

large proportion of linoleic acid (18:2ω6), the dietary essential PUFA which is found in highest concentration in terrestrial plant sources, especially vegetables, nuts, and seeds.

The fatty acid composition of the non-esterified fatty acid fraction was similar in both species to that of the triacylglycerol fraction except that the proportion of arachidonic acid (20:4ω6) was greater in non-esterified fatty acid, especially in black bears (Table 5.3). This was more likely an effect of the conversion of linoleic (18:2ω6) to arachidonic acid through desaturase and elongase enzyme activity rather than an effect of diet; in both species the difference in the proportions of linoleic acid between non-esterified fatty acid and triacylglycerol fractions corresponded approximately with the difference in the proportions of arachidonic acid between fractions, but in opposite directions. Because non-esterified fatty acids are primarily products of lipolysis in adipose tissue, their composition of fatty acids is influenced by desaturase and elongase enzyme activity and fatty acid biosynthesis (lipogenesis), as well as by diet.

Differences in the fatty acid composition of phospholipid between species were similar to the differences described for the other fractions (Table 5.4 and Fig. 5.2). However, the fatty acid composition of the phospholipid fraction in both species differed from that of the other lipid fractions, primarily as a result of greater proportions of arachidonic (20:4ω6) and stearic (18:0) acids, and lower proportions of myristoleic acid (14:1ω5) and ω7 fatty acids. The fatty acid composition of phospholipid typically is similar between plasma and tissue (Holman 1986), and is dictated by the requirement of cell membranes and the immune system to maintain normal function. The results from polar and black bears suggest that diet did not influence the fatty acid composition of the phospholipid fraction as much as it may have influenced that of the other fractions, and imply that dietary and endogenously-synthesized fatty acids were selectively used during phospholipid biosynthesis.

5.4.2 Plasma and adipose tissue fatty acid composition in feeding and fasting polar bears

There was no evidence of EFA deficiency during prolonged fasting in polar bears (Tables 5.2 to 5.5 and Figs. 5.3 and 5.5). Essential fatty acid deficiency typically is characterized by low

proportions of ω6 and ω3 fatty acids and concurrent high proportions of monoenoic acids and the polyenoic products of oleic acid (18:1ω9), i.e., 20:2ω9 and 20:3ω9 (Holman, 1968). The TRI:TET ratio (20:3ω9 + 20:4ω6) was originally proposed as an index of EFA status sensitive to deficiency of linoleic acid (18:2ω6) (Holman 1960), and is useful as a measure of simple nutritional ω6 fatty acid deficiency. The E:NE ratio also was used in this study as an index sensitive to change in the total content of EFA's and their products, relative to the content of all other fatty acids. In polar bears, the TRI:TET ratio did not differ significantly between feeding and fasting bears in the plasma non-esterified fatty acid and phospholipid fractions, or in the adipose tissue triacylglycerol. The E:NE ratio did not differ significantly between feeding and fasting bears in any plasma lipid fraction. Thus, the polar bears were able to maintain adequate amounts of EFA's during prolonged fasting.

There also was no evidence of EFA deficiency during pregnancy and lactation in fasting polar bears (Table 5.6). Five of the 82 fasting polar bears sampled in this study were adult females accompanied by new-born cubs and were captured after 8-9 months of fasting, during which time all of post-implantation gestation and early lactation occurred. Although there were differences in the proportions of some individual EFA's, there were no significant differences in the TRI:TET, E:NE, and ω6:ω3 ratios in any plasma lipid fraction between these 5 bears and the other 77 fasting polar bears. This implies that fasting female polar bears also were able to meet their additional requirement for EFA's for pregnancy and lactation without showing any evidence of EFA deficiency.

There are two possible mechanisms by which polar bears were able to maintain adequate amounts of EFA's during prolonged fasting; either by storage of diet-derived EFA's in adipose tissue or by biosynthesis of linoleic (18:2ω6) and α-linolenic (18:3ω3) acids. The storage and conservation of EFA's in adipose tissue during feeding for later use in phospholipid and eicosanoid biosynthesis during fasting has been suggested to occur in obese humans undergoing rapid weight loss while consuming very-low-calorie diets (Phinney et al. 1990, Phinney et al. 1991). Adult polar bears typically accumulate large amounts of body fat while feeding during late spring (Ramsay and Stirling 1988, Atkinson et al. 1996a) and may have been able to store a sufficient quantity of linoleic and α-linolenic acids in adipose tissue while feeding to maintain their EFA requirements while fasting. The

Table 5.6 Proportions and indices of essential fatty acids in the plasma lipid fractions of fasting female polar bears, that were accompanied by new-born cubs, and all other fasting adult polar bears.[a]

Classification	Fasting female bears with cubs (n = 5)	Other fasting adult bears (n = 77)	Comparison[b]
1. Triacylglycerol			
18:2ω6	7.39±0.25	6.59±0.16	ns
20:4ω6	2.50±0.19	1.96±0.05	**
18:3ω3	0.72±0.03	0.94±0.04	ns
TRI:TET ratio	0.006±0.006	0.054±0.010	ns
E:NE ratio	0.70±0.07	0.61±0.01	ns
ω6:ω3 ratio	0.37±0.03	0.36±0.01	ns
2. Non-Esterified Fatty Acid			
18:2ω6	3.81±0.40	3.80±0.16	ns
20:4ω6	2.29±0.63	3.23±0.26	ns
18:3ω3	0.53±0.01	0.79±0.03	***
TRI:TET ratio	0.037±0.009	0.031±0.005	ns
E:NE ratio	0.37±0.03	0.45±0.01	ns
ω6:ω3 ratio	0.36±0.05	0.39±0.02	ns
3. Phospholipid			
18:2ω6	8.23±0.59	5.95±0.24	**
20:4ω6	12.92±0.85	12.64±0.29	ns
18:3ω3	0.03±0.01	0.10±0.02	ns
TRI:TET ratio	0.014±0.001	0.017±0.001	ns
E:NE ratio	0.67±0.06	0.53±0.01	ns
ω6:ω3 ratio	1.69±0.12	1.75±0.07	ns

[a] Proportions expressed as mean percentage of total fatty acids in lipid fraction ± standard error of mean.

[b] Student's t-test was used to compare between groups. Significance was assigned at $p \leq 0.017$ based on the Dunn-Šidák method (Ury 1976) and is indicated by "ns" for non-significance, ** for $p \leq 0.017$, and *** for $p \leq 0.001$.

observation that proportions of linoleic and α-linolenic acids in the adipose tissue triacylglycerol of fasting polar bears were similar, or even slightly increased, from the proportions in feeding bears supports this explanation. A less probable explanation is that polar bears were able to synthesize linoleic and α-linolenic acids, as this would require the presence of Δ12 and Δ15 desaturases. These enzymes occur in plants and certain insects, but are not known to exist in mammals (Hadley 1985).

Fasting polar bears had significantly lower proportions of eicosapentaenoic acid (20:5ω3) in all plasma lipid fractions, and in adipose tissue triacylglycerol, than did feeding polar bears (Tables 5.2 to 5.5). These results suggest that fasting polar bears may have selectively removed eicosapentaenoic acid from plasma and adipose tissue through its conversion to docosahexaenoic acid (22:6ω3), possibly to maintain normal synthesis of eicosanoids. Eicosapentaenoic acid inhibits the conversion of arachidonic acid to the biologically active eicosanoids (prostaglandins, prostacyclin, thromboxanes, lipoxins, leukotrienes, and related compounds). In humans consuming diets rich in eicosapentaenoic acid, platelet aggregation becomes diminished and bleeding times prolonged (Dyerberg and Bang 1979) and sterility may develop due to a decrease in the prostaglandin content of seminal fluid and the consequent disappearance of spermatozoa (Sinclair 1982).

5.4.3 Plasma and adipose tissue fatty acid composition in feeding and fasting black bears

The proportions of linoleic (18:2ω6) and α-linolenic (18:3ω3) acids in most lipid classes in plasma, and in adipose tissue triacylglycerol, were lower in fasting bears than in feeding bears (Tables 5.2 to 5.5 and Figs. 5.4 and 5.5). Furthermore, the TRI:TET and E:NE ratios of fasting bears were generally lower than those of feeding bears. However, despite the lower proportions of linoleic and α-linolenic acids, the proportion of arachidonic acid (20:4ω6) in the plasma phospholipid of fasting bears was greater than in feeding bears. This suggests that the relatively low proportion of EFA in fasting black bears was probably of little physiological significance and unlikely to have affected the normal synthesis of eicosanoids.

The reason why the proportion of EFA in plasma and adipose tissue was similar between feeding and fasting polar bears, but lower in fasting black bears than in feeding black bears, was not determined. It is possible that the complete commercial ration consumed by the captive black bears did not contain linoleic (18:2ω6) and α-linolenic (18:3ω3) acids in sufficient quantity to prevent the proportion of EFA stored in body fat from decreasing during winter dormancy. Alternatively, it may be that polar bears are better able to maintain EFA's during prolonged fasting than are black bears, either as a consequence of their different diets or as a consequence of differences in their metabolism of fatty acids. Determination of the fatty acid composition of plasma and adipose tissue from free-ranging black bears that are consuming a natural diet would help to evaluate the role of diet in maintaining EFA's during prolonged fasting.

6. ASPECTS OF CARBOHYDRATE METABOLISM AND REGULATION IN FREE-RANGING ADULT POLAR BEARS

6.1 Introduction

High fat and low carbohydrate diet, seasonal obesity, and prolonged fasting are the usual circannual dietary habits of the polar bear (*Ursus maritimus*). In humans and laboratory animals, high dietary fat is an important factor contributing to the development of obesity (Salmon and Flatt 1985; Schutz et al. 1989, reviewed in Hill and Peters 1998). Obesity, in turn, is associated with hyperglycemia, insulin resistance, and type II diabetes mellitus (Randle et al. 1965, Boden et al. 1983, reviewed in Kissebah and Krakower 1994). High dietary fat also contributes directly to the development of insulin resistance and type II diabetes mellitus in non-obese individuals through the progressive enhancement of pyruvate dehydrogenase kinase activity in oxidative tissues (Sugden et al. 1995). In both normal-weight and obese humans, prolonged fasting results in the mobilization of fatty acids and glycerol from adipose tissue, an adaptive response initiated by low dietary carbohydrate and the concurrent decline in blood glucose (Klein et al. 1990, Klein and Wolfe 1992).

The regulation and metabolism of carbohydrate by polar bears during feed and fast has not been studied. However, the annual cycle of high fat diet, seasonal obesity, and prolonged fasting suggest that polar bears do not suffer the severe health effects experienced by obese or starving humans. In this study, plasma concentrations of glucose, cortisol, triiodothyronine, thyroxine, insulin, and glucagon were measured in free-ranging polar bears captured throughout the year to determine the association of these plasma constituents with nutritional state (feeding versus fasting), sex (female versus male), and body condition (lean versus fat). Plasma insulin and glucagon concentrations also were measured in polar bears following the intravenous administration of glucose

to compare changes in the plasma concentrations of these key glucoregulatory hormones among bears of different nutritional state and body condition.

6.2 Materials and methods

One hundred and fifty-one, free-ranging, adult polar bears were captured from August 1994 to November 1996. All bears were located by helicopter and chemically immobilized with Telazol® (8 to 12 mg/kg intramuscularly) using remote injection equipment (Stirling et al. 1989). Sixty-eight bears were captured during spring (April and May) while they were hunting seals on arctic sea-ice near Resolute Bay, Nunavut, Canada (74°00' to 76°00'N and 93°00' to 100°50'W). Eighty-five adult polar bears were captured during summer, fall, and winter (July to November, and March) while they were fasting on land along the west coast of Hudson Bay near Churchill, Manitoba, Canada (57°00' to 58°50'N and 92°25' to 94°15'W). The polar bears captured during March were comprised of six adult females accompanied by new-born cubs that had emerged recently (within days) from their snow dens after 8 to 9 months of fasting that included pregnancy and early lactation.

Bears were weighed (± 0.5 kg) with an electronic load scale while suspended in a sling. Body length was measured by positioning bears in sternal recumbency and measuring the straight-line distance (± 0.5 cm) between the distal tips of nose and tail. Body condition of captured bears was quantified by a body condition index (BCI) based on the relationship between body mass and length (see Chapter 2). Rectal temperatures were recorded with a digital thermometer (AMG Medical, Montreal, Quebec, Canada) at 10 minute intervals throughout the 3-hour handling period. Ages of bears previously captured were obtained from a national database (Canadian Wildlife Service, Edmonton, Alberta). The ages of bears not previously captured were determined by extracting, sectioning, staining, and counting cementum annuli on one first premolar tooth (Stoneberg and Jonkel 1966). Bears 5 years of age or older were classified as adults (Lunn et al. 1997).

Twenty milliliters of blood was collected from the jugular vein into Vacutainer® tubes containing sodium heparin (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA).

Blood samples were cooled immediately following collection and were centrifuged within 12 hours to collect and freeze plasma. Plasma glucose, cortisol, and thyroid hormone concentrations were determined by the Clinical Pathology Laboratory at the Western College of Veterinary Medicine, University of Saskatchewan, Saskatchewan, Canada. The glucose was measured using an Abbott Spectrum® Series II biochemistry analyzer (Abbott Laboratories Diagnostics Division, Abbott Park, Illinois 60064, USA) and plasma concentrations of total cortisol, triiodothyronine, and thyroxine were measured directly, without extraction, using a fluorescence polarization immunoassay (TD_X System, Abbott Laboratories, Irving, TX, USA). Plasma insulin and glucagon concentrations were measured by Bio-Stress Research (Waterloo, Ontario, Canada) using commercially available radioimmunoassay kits (Inter-Medico, Willowdale, Ontario, Canada). Both assays were confirmed to give valid results using the procedures of parallelism and quantitative recovery.

A total of 10 polar bears captured during different seasons were administered glucose to determine and compare changes in plasma glucose, insulin, and glucagon concentrations in association with nutritional state and body condition. Glucose (Dextrose 50%, Vetoquinol N.-A. Inc., Lavaltrie, Quebec, Canada) was administered by slow bolus injection, via an intravenous catheter, into the jugular vein at 500 mg glucose per kg body mass. A baseline blood sample was drawn immediately prior to glucose administration and samples were taken at 5, 10, 15, 30, 45, 60, 90, 120, and 150 minutes following glucose administration. Blood samples were centrifuged, the plasma frozen, and plasma glucose, insulin, and glucagon were measured as described above.

Samples of frozen liver collected during 1993-94 from nine feeding adult male polar bears killed by Inuit hunters during spring, and from three fasting adult polar bears (two females and one male) killed by conservation enforcement officers during fall, were obtained to measure hepatic glycogen content. All liver samples were collected and frozen in liquid nitrogen at the time of death. To measure the glycogen concentration, 400 mg of each sample was homogenized and acidified with 100 µl of 0.6 N perchloric acid (PCA). The acidified homogenate was neutralized with 1:1 v/w of 1 M $KHCO_3$, and the glucose concentration was measured with an Abbott Spectrum® Series II biochemistry analyzer (Abbott Laboratories Diagnostics Division, Abbott Park, Illinois 60064,

USA). An aliquot (~50 µl) of homogenate was hydrolyzed with 500 µl of amyloglucosidase (Sigma Chemical Co., St. Louis, Missouri, USA) in 500 µl of acetate buffer, and incubated at 40°C for 2 hours. The hydrolyzed homogenate was acidified with 0.6 N PCA (25µl) and centrifuged at 14,000g for 10 minutes. The supernatant was collected, its glucose concentration was measured, and the glucose contributed by the amyloglucosidase was subtracted to give the glucose concentration for the hydrolyzed supernatant. Glycogen concentration (µmol glucosyl units/g liver weight) was calculated by subtracting the concentration of glucose in the homogenate from that in the hydrolyzed supernatant.

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, Illinois, USA). Two-way analysis of variance (ANOVA) was used to determine differences in plasma glucose, hormones, and the insulin-to-glucagon molar ratio (I/G molar ratio; Unger 1971) among nutritional states (feeding, fasting, and fasting that included pregnancy and early lactation), and between sexes (Zar 1996). The body condition index (BCI) and drug dosage (mg/kg) were included as covariates. Pairwise associations between plasma constituents and the BCI were measured using Pearson (r) and partial ($r_{partial}$) correlation analyses. Repeated measures ANOVA was used to determine the significance of changes over time in the plasma concentrations of glucose, cortisol, insulin, and glucagon, and in the I/G ratio, of individual bears (Zar 1996). Tukey's Honestly Significant Difference test was used to make multiple comparisons among means (Zar 1996). The Mann-Whitney U-test was used to compare liver glycogen concentration between feeding and fasting bears. For all statistical tests, significance was assigned when the probability of a Type I error was equal to or less than 0.05. All results are reported as mean ± standard error (SE).

6.3 Results

The plasma concentrations of glucose, cortisol, insulin, and glucagon, and the insulin:glucagon (I/G) molar ratio, covaried significantly with the Body Condition Index (BCI) of individual polar bears (Table 6.1). The pairwise correlations of these plasma constituents with the BCI also were significant with the association being negative for glucose (r = -0.26, $p < 0.01$, n =

Table 6.1 Plasma concentrations of glucose and selected hormones, the I/G molar ratio, and rectal temperature in free-ranging adult polar bears captured during feeding (spring) and fasting (summer-fall), and during fasting that included with pregnancy and early lactation (winter).[a]

Plasma constituent	Feeding (spring)		Fasting (summer-fall)		Fasting+[b] (winter)	Factor or Covariate			
	female (n = 7-38)	male (n = 7-30)	female (n = 6-49)	male (n = 10-28)	female (n = 3-6)	nutritional state	sex	nutritional state × sex	BCI
glucose (mmol/L)	5.8±0.20 (3.4-8.8)	5.8±0.19 (4.0-7.7)	5.6±0.16 (3.4-8.2)	5.9±0.16 (4.8-8.0)	8.3±0.94 (7.0-9.3)	***	ns	ns	**
cortisol (nmol/L)	479±45.4 (170-1010)	354±32.4 (114-942)	558±65.1 (204-1153)	390±43.7 (77-724)	801±174.6 (139-1423)	ns	*	ns	*
insulin (pmol/L)	63±18.5 (20-168)	154±81.6 (20-637)	84±17.8 (18-309)	98±25.1 (45-274)	75±14.6 (30-125)	ns	ns	ns	**
glucagon (µg/L)	47±8.3 (18-75)	50±9.9 (17-84)	39±3.3 (15-61)	43±7.1 (19-74)	76±8.5 (46-95)	**	ns	ns	*
I/G molar ratio	5.6±1.45 (1.1-12.4)	12.7±4.60 (1.0-34.6)	9.3±2.24 (1.4-32.3)	9.3±3.75 (3.0-38.8)	3.5±0.56 (1.2-4.9)	ns	ns	ns	***
triiodothyronine (nmol/L)	0.90±0.082 (0.25-2.24)	0.64±0.049 (0.25-1.00)	0.31±0.035 (0.25-0.44)	0.56±0.081 (0.25-1.39)	1.60±0.125 (1.40-183)	***	ns	**	ns
thyroxine (nmol/L)	15.4±0.84 (7.0-30.0)	13.6±0.42 (10.0-20.0)	7.4±1.40 (4.5-12.3)	13.7±1.25 (4.4-24.0)	18.0±1.57 (15.1-20.5)	*	ns	***	ns
rectal temperature[c] (°C)	37.9±0.46 (34.1-39.7)	38.3±0.29 (36.4-39.8)	36.8±0.20 (34.1-39.3)	36.8±0.29 (33.9-39.8)	37.6±0.29 (36.2-38.3)	***	ns	ns	ns

[a] Values reported as mean ± SE with range presented in parentheses. Statistical comparison made by two-way ANOVA with nutritional state and sex as the factors, nutritional state × sex as the interaction, and the Body Condition Index (BCI) as the covariate. Significance indicated by "ns" for non-significance, * for $p \leq 0.05$, ** for $p \leq 0.01$, and *** for $p \leq 0.001$.

[b] Adult female bears captured during winter that were accompanied by new-born cubs, and that had emerged recently (within days) from their snow dens.

[c] Values reported for rectal temperature were recorded at 30 minutes following capture.

109), cortisol (r = -0.35, $p < 0.01$, n = 82), and glucagon (r = -0.32, $p < 0.05$, n = 45), and positive for insulin (r = 0.37, $p < 0.05$, n = 45) and the I/G molar ratio (r = 0.55, $p < 0.001$, n = 45). Plasma glucose, glucagon, and triiodothyronine (T3) concentrations were affected by nutritional state, with fasting females captured during winter having significantly higher values than feeding or fasting bears captured at other times of the year. In feeding polar bears, plasma T3 and thyroxine (T4) values were significantly greater in females than in males. The pattern was opposite in fasting polar bears captured during summer-fall, with thyroid hormone values greater in males than in females. Plasma T3 and T4 values, however, did not differ between feeding and fasting males. Rectal temperature values at 30 minutes following capture were significantly lower in fasting bears captured during summer-fall than in bears captured during other times of the year. Plasma cortisol concentration was significantly higher in females than males, irrespective of nutritional state. There were no significant correlations of drug dosage with the concentrations of any of the plasma constituents, the I/G molar ratio, or rectal temperature.

Throughout the 3-hour period in which captured bears were handled and sampled, the plasma glucose concentration of fasting females captured during winter was significantly higher (F = 3.87, $p < 0.05$) than that of other feeding or fasting bears, but it increased significantly (repeated measures ANOVA; F = 4.63, $p < 0.001$, n = 54) in all polar bears during handling, irrespective of their nutritional state (Fig. 6.1). The plasma glucose concentration also was measured in five fasting bears at 15 minute intervals for one hour following capture, but values in these bears increased only slightly and not significantly (F = 2.33, p = 0.13) (Fig. 6.2). The plasma cortisol concentration, however, decreased significantly (F = 19.34, $p < 0.001$) over the same time.

Plasma concentrations of glucose, insulin, and the I/G molar ratio in ten polar bears changed significantly (repeated measures ANOVA; F = 6.19 to 83.54, $p < 0.001$) over a 150 minute period following the intravenous administration of glucose (Fig. 6.3). However, the plasma glucagon concentration did not change significantly over this time (F = 1.03, p = 0.42). The plasma concentrations of glucose and glucagon were significantly affected by nutritional state, with the one adult female captured during winter (while accompanied by cubs) having higher values than other

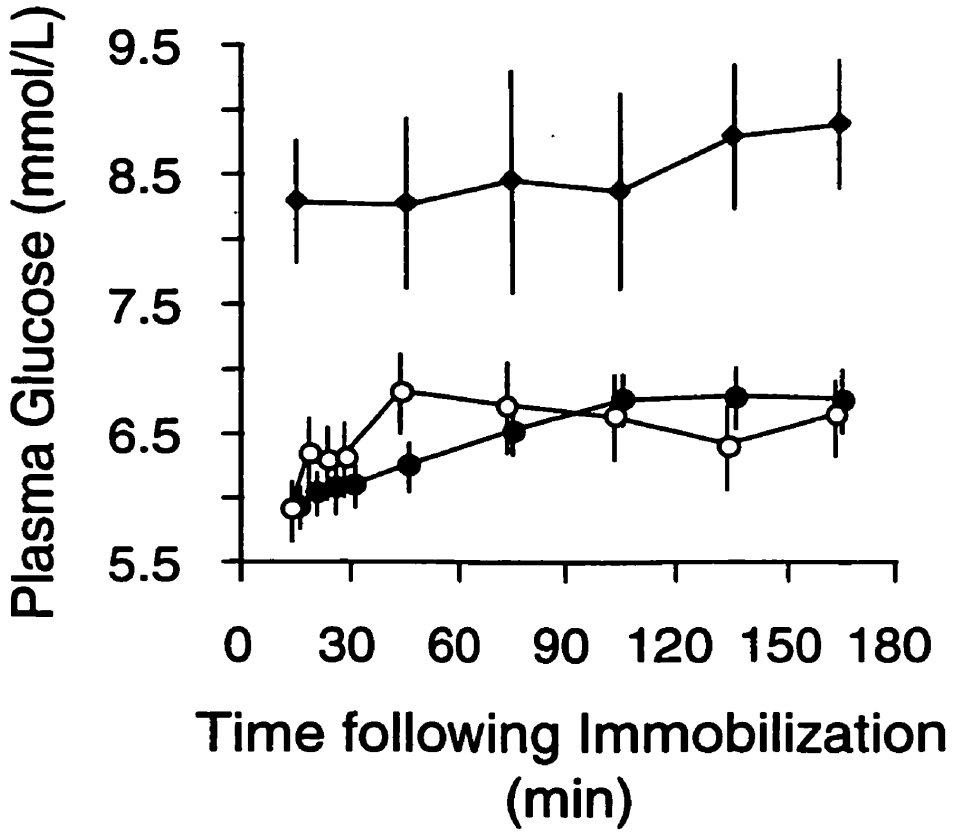


Figure 6.1 Plasma glucose concentrations of polar bears at 15, 20, 25, 30, 45, 75, 105, 135, and 165 minutes following chemical immobilization. Values are presented as the mean ± standard error bars for polar bears captured during feeding (spring; O ; $n = 25$) and fasting (summer-fall; ● ; $n = 24$), and during fasting that included pregnancy and lactation (winter; ◆ ; $n = 5$). Bears captured during winter were adult females that were accompanied by newborn cubs, and that had emerged recently (within days) from their snow dens. Statistical comparison by repeated measures ANOVA indicated a significant ($F = 4.63$, $p < 0.01$) increase over time in the plasma glucose concentration of bears, irrespective of their nutritional state.

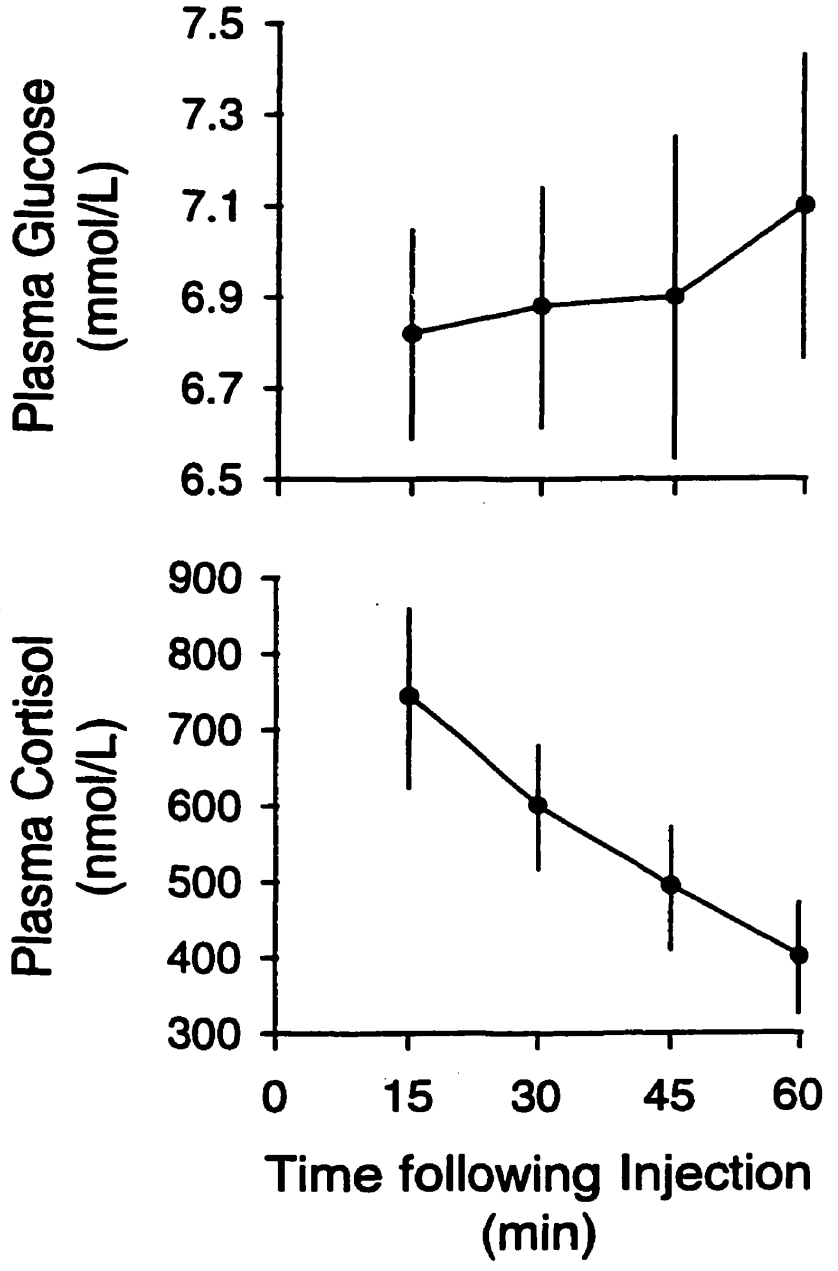


Figure 6.2 Plasma glucose and cortisol concentration of five fasting polar bears (three females and two males) at 15, 30, 45, and 60 minutes following intramuscular administration of the anesthetic drug. Values are presented as the mean ± standard error bars. Statistical comparisons by repeated measures ANOVA indicated no significant ($F = 2.33$, $p = 0.13$) change over time in plasma glucose concentration, but a significant ($F = 19.34$, $p < 0.001$) decrease over time in plasma cortisol concentration.

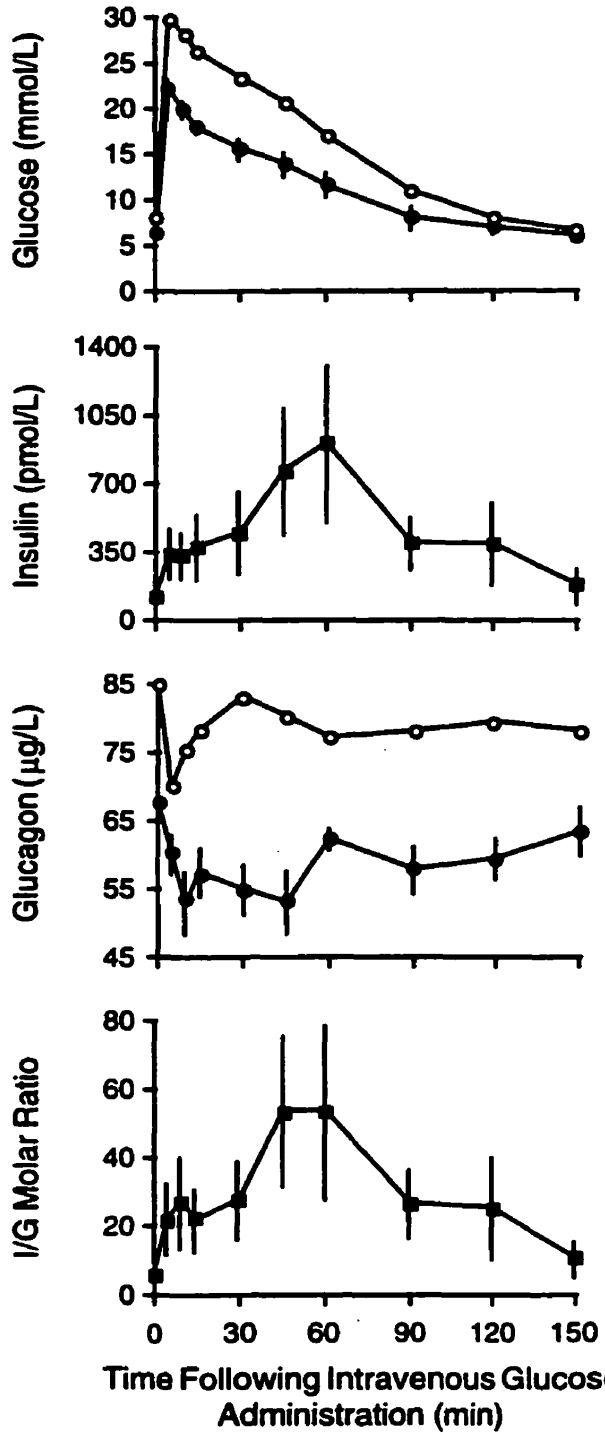


Figure 6.3 Plasma glucose, insulin, and glucagon concentrations, and the I/G molar ratio in 10 polar bears following intravenous administration of glucose (500 mg/kg). Values are plotted as the mean with vertical lines indicating the standard error of the mean for nine polar bears captured during spring or summer-fall (●), a single female polar bear captured during winter within days after emerging from her snow den with two new-born cubs (O), and all 10 polar bears combined (■). Statistical comparisons by repeated measures ANOVA indicated significant changes over time in the plasma concentrations of glucose and insulin, and the I/G molar ratio ($F = 6.19$ to 83.54, $p < 0.001$), but not in the plasma glucagon concentration ($F = 1.03$, $p = 0.42$).

bears for both constituents (glucose: $F = 8.37$, $p < 0.05$; and glucagon: $F = 2.91$, $p < 0.05$) at most sampling times. The plasma insulin concentration and the I/G molar ratio covaried significantly (insulin: $t = 3.94$, $p < 0.01$; I/G molar ratio: $t = 3.47$, $p < 0.05$) with the body condition of the individual bears. Of two feeding bears captured during spring, an adult male with a BCI value of 0.27 (approximately 29% body fat; see Chapter 2) had markedly higher plasma insulin concentrations and I/G molar ratio, and larger changes in plasma glucagon concentration, than did an adult female with a BCI value of -1.89 (approximately 11% body fat) (Fig. 6.4).

Associations between the plasma insulin concentration and the concentrations of other plasma constituents were determined, while controlling for variation in the BCI value, using plasma values measured concurrently during two other studies (see Chapters 3 and 4). Insulin values were positively correlated with the plasma glucose concentration ($r_{partial} = 0.40$, $p < 0.01$, $n = 45$), but not with the plasma concentrations of alanine ($r_{partial} = -0.28$, $p = 0.28$, $n = 20$), non-esterified fatty acid ($r_{partial} = 0.24$, $p = 0.30$, $n = 20$), glycerol ($r_{partial} = -0.13$, $p = 0.56$, $n = 20$), or total ketone bodies (acetoacetate + β-hydroxybutyrate; $r_{partial} = 0.07$, $p = 0.78$, $n = 20$).

The liver glycogen concentration for three fasting polar bears captured during fall was signficantly greater than that for nine feeding polar bears captured during spring ($U = 2.31$, $p < 0.05$; Fig. 6.5b).

6.4 Discussion

In this study, body condition was defined as the abundance of potential metabolic energy stored in an animal's tissues (primarily adipose tissue and skeletal muscle) relative to its body size. The body condition index (BCI) was developed to provide an estimate of the body condition of individual polar bears relative to other bears in a sample population of 1072 animals (see Chapter 2). Although the BCI is an index without units, it can be used in conjunction with the body length of a bear to predict its mass of adipose tissue, skeletal muscle, or both tissues. The range of BCI values is distributed normally around a mean value of zero, with positive values indicating a larger

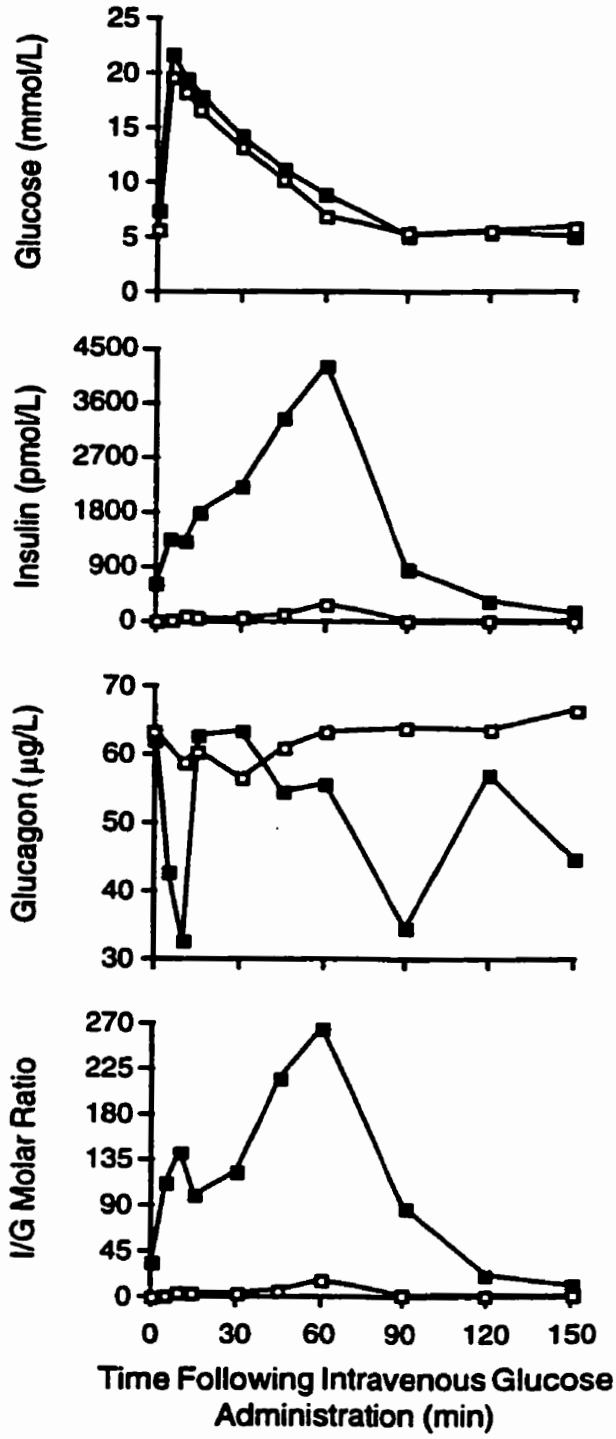
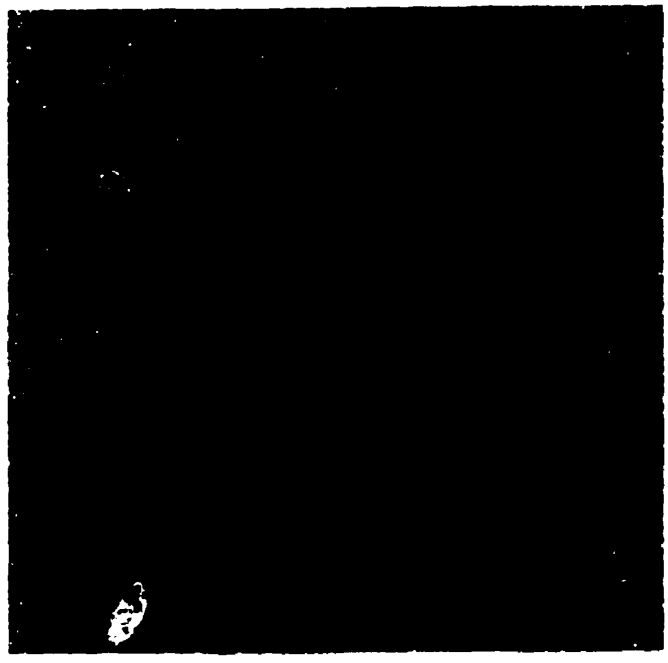


Figure 6.4 Plasma glucose, insulin, and glucagon concentrations, and the I/G molar ratio in two polar bears of different body condition following intravenous administration of glucose (500 mg/kg). Individual values at different sampling times are plotted for an adult male that was fat (BCI = 0.27, which indicates the body fat content was approximately 29% of total body mass; ■) and an adult female that was lean (BCI = -1.89, which indicates the body fat content was approximately 11% of total body mass; □).

a)



b)

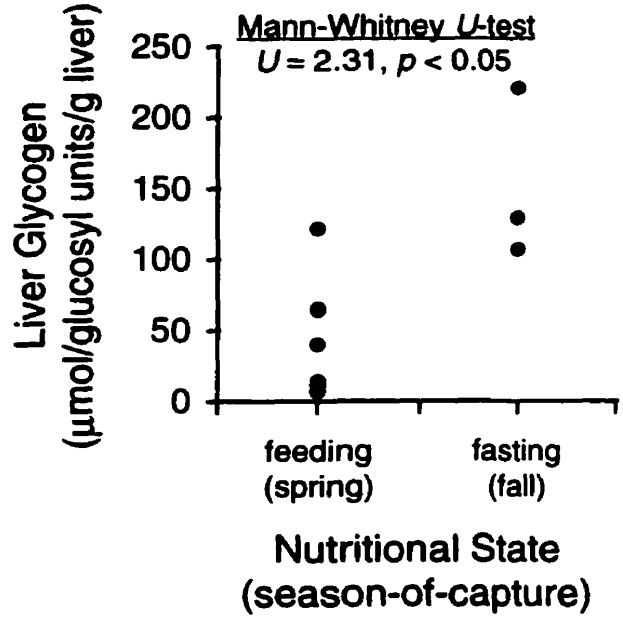


Figure 6.5 Glycogen storage in the liver of fasting polar bears. a) A histologic section of liver collected from an adult male polar bear following 4 months of fasting contains large stores of glycogen. The glycogen appears deep pink following staining of the section with Periodic acid-Schiff (PAS) stain. Magnification - 10x objective. b) Liver glycogen concentration in nine feeding polar bears captured during spring and three fasting polar bears captured during fall.

availability of potential metabolic energy and negative values indicating a smaller availability of potential metabolic energy, relative to the mean body condition of the sample population.

Body condition was an important factor influencing the metabolism and regulation of glucose in polar bears. Plasma concentrations of glucose, cortisol, and glucagon were lower, and the plasma insulin concentration and insulin:glucagon (I/G) molar ratio were higher in fat bears (BCI > 0) when compared with lean bears (BCI ≤ 0) (Table 6.1). Furthermore, changes in the plasma insulin concentration and I/G molar ratio of bears following intravenous administration of glucose (glucose tolerance test) were greatly exaggerated in fat bears relative to the changes observed in lean bears (Fig. 6.4). Together, these results indicate that greater body condition in polar bears was associated with either a large amount of insulin secretion, or a slow rate of insulin clearance from plasma, or a combination of both factors. Because changes in plasma glucose concentration following the intravenous administration of glucose were similar among bears that varied widely in body condition (BCI values from -1.97 to +0.72, or from approximately 10% to 37% body fat), the high insulin values in fat bears were more likely affected by a slow rate of insulin clearance than by a large amount of insulin secretion. Insulin clearance is a function of the uptake of insulin from plasma by insulin receptors on target cells and a slow rate of insulin clearance often reflects a low sensitivity of tissues (e.g., due to a low number of receptors or a decrease in the affinity of receptors) to insulin, a condition termed insulin resistance (Cotran et al. 1999). The results of this study suggest that insulin resistance is a normal response of polar bears to alterations in stored potential metabolic energy, waxing or waning as their body condition increases or decreases.

In contrast to humans, insulin resistance in polar bears is not known to be associated with metabolic disease, e.g., type II diabetes mellitus, dyslipidemia. This suggests the effects of insulin resistance are different between these species. In humans, plasma concentrations of glucose, alanine, non-esterified fatty acid, glycerol, and total ketone bodies (acetoacetate and β-hydroxybutyrate) are higher in obese than in normal-weight subjects, and are significantly associated with both the plasma insulin concentration and the degree of obesity (Golay et al. 1986, Robertson et al. 1991). The metabolic features underlying these plasma differences are a decreased sensitivity of peripheral

tissues to insulin-stimulated glucose uptake, a decreased sensitivity of liver to insulin-inhibited gluconeogenesis, and a reduction in the suppression of fatty acid metabolism by insulin (Groop et al. 1989, Groop et al. 1992, Ferrannini 1995). In polar bears, the plasma glucose concentration of fat bears was slightly lower than that of lean bears, and the BCI was not a signficant covariate with the plasma concentrations of alanine, non-esterified fatty acid, glycerol, or total ketone bodies (see Chapters 3 and 4). Furthermore, the plasma insulin concentration was only associated with the plasma glucose concentration and not with the concentrations of other plasma constituents. The metabolic features of polar bears that account for these differences from humans are not known. Nevertheless, these results suggest that insulin resistance in polar bears is associated with decreased use of glucose by peripheral tissues without significant alterations in fatty acid metabolism. If this is the case, polar bears may be increasingly able to spare glucose (and, therefore, protein and amino acids), while using fatty acids for energy, as their quantity of body fat increases.

The plasma glucose concentrations of bears in this study were high when compared to normal reference values for humans and domestic mammals (Tietz et al. 1990, Duncan et al. 1994), and exceeded the 95% confidence interval for the blood glucose concentration of 73 other mammalian species when scaled to body mass (Umminger 1975). It is likely that these high values were associated with acute stress arising from the capture procedure, as evidenced by the concurrent high concentrations of plasma cortisol at the initial sampling time relative to later sampling times.

The finding that the high concentrations of plasma glucose persisted, and often increased, in polar bears throughout the 3 hour handling period that followed their capture (Fig. 6.1), irrespective of the differences in body condition among bears, is difficult to explain. It is most likely that the persistence of high glucose values reflected both a delayed secretion of insulin and a slow clearance of glucose from plasma, rather than a high rate of gluconeogenesis induced by stress or possibly anesthesia. This is supported by observations that, following the administration of glucose intravenously, the rise in plasma insulin concentration and the return of plasma glucose concentration to baseline values in polar bears (that varied widely in body condition) was prolonged (Figs. 6.3 and 6.4) relative to rates typically encountered in humans and domestic mammals [e.g., in domestic

species, insulin concentrations typically peak at 5 minutes and peak glucose values decrease by one-half within 45 minutes (Seltzer et al. 1967, Duncan et al. 1994)]. This suggests that insulin secretion and clearance may normally be slow in polar bears, relative to other mammals, independent of the effect of body condition. In regard to stress effects, the plasma cortisol concentration of bears decreased by approximately 40-50% during the first hour following capture (Fig. 6.2). Although the physiological effects of cortisol may continue well after its plasma concentration decreases, plasma glucose concentration typically returns to pre-stress values in response to a rise in plasma insulin concentration (Goldstein et al. 1993). The anesthetic drug Telazol® has not affected plasma glucose concentrations in a variety of mammals (Van Heerden et al. 1991, Lin et al. 1993, Peinado et al. 1993). Furthermore, the dosage of Telazol® administered to polar bears in this study did not correlate with their plasma glucose concentration.

Relative to body condition, nutritional state (feeding or fasting) appeared to have little effect on the regulation of plasma glucose in polar bears, but did have a significant effect on the total energy metabolism of polar bears. In females captured shortly after emerging from their winter dens, the plasma concentrations of glucose, glucagon, cortisol, and triiodothyronine (T3) were high, and the I/G molar ratios were low, relative to bears captured during other seasons (Table 6.1 and Fig. 6.3). Furthermore, the plasma concentration of thyroxine (T4) and the rectal temperatures of fasting females captured during winter were high relative to fasting bears captured during summer-fall, but similar to values measured in feeding bears. Together, these results suggest females emerging from their winter dens had high metabolic rates (relative to other fasting bears) and were mobilizing their energy stores. Higher plasma glucagon values combined with lower I/G molar ratios would result in a higher rate of gluconeogenesis and the mobilization of glucose stored in glycogen (glycogenolysis) (Unger 1971). Higher plasma cortisol values would contribute to the permissive role this hormone plays in affecting the action of glucagon during fasting (Ganong 1995). And lastly, higher plasma concentrations of T3 and T4 and a higher rectal temperature in fasting females captured during winter indicates they had a higher metabolic rate than fasting bears captured during summer-fall (Ganong 1995).

The demand on body energy stores is greatest for female polar bears during summer-fall (in association with pregnancy and lactation) when food is less available or not available at all. Thus, a depression of metabolic activity as indicated by their lower values for plasma T3 and T4 and rectal temperature (relative to feeding females) conserves stored body fuels (fat and protein) during prolonged fasting (Hochachka and Guppy 1987). In males, the demand on body energy stores is probably greatest during spring (in association with breeding activity) when, despite the availability of food, they will fast intermittently while seeking a mate. The lower T3 and T4 values in feeding males relative to feeding females may have reflected a slower metabolic rate. The fact, however, that rectal temperatures were similar between feeding males and females, and that normal sexual function is dependent on normal thyroid secretion (Guyton 1986), suggest any difference in metabolic rate between feeding males and females probably was small. In contrast, fasting males had higher plasma T3 and T4 values than fasting females. Rectal temperatures, however, were similar between males and females, but lower than the values recorded in feeding bears. Together, these results suggest that, as in female bears, the metabolic rate of fasting males was slower than that of feeding males, but the magnitude of difference in metabolic rates between feeding and fasting was probably not as extreme in males as it was in females. Similar interactions between sex and nutritional state affected the plasma concentration of total amino acids (Chapter 3) and the plasma concentrations of acyl-carnitine and free carnitine (Chapter 4) in polar bears.

During a routine histologic examination of tissues collected from a killed adult male polar bear, an abundance of glycogen was observed in sections of liver (Fig. 6.5a). Because the bear had been killed during fall after approximately 4 months of fasting, the presence of glycogen was an unexpected finding and prompted the subsequent determination of glycogen concentrations in the livers of feeding and fasting polar bears (Fig. 6.5b). The presence of liver glycogen following prolonged fasting, coupled with the finding that rates of insulin secretion and clearance appear to be slow in polar bears, suggest that polar bears are not as dependent on glucose for energy as are other mammals and, as a consequence, are more lax in regulating their body glucose stores.

7. GENERAL DISCUSSION

7.1 Similar scenarios, different outcomes revisited

Why did the loss of body weight lead to the death of Janice the computer artist, but appear to be of little consequence to the health of the polar bear X09111? From the large amount of research that has been carried out on the need for food in humans, we can speculate on the likely sequence of biochemical and physiological events that occurred in Janice, eventually leading to her death. At the outset, it is important to realize that not only did Janice drastically decrease her daily consumption of calories for a prolonged period, but her daily physical activity also increased substantially. The net effect of these lifestyle changes was that Janice became heavily dependent on the chemical constituents of her body to provide the fuel necessary to meet her daily energy demand.

During the first week of her diet, it was likely that Janice completely depleted the glycogen that was stored in her liver. This stage is termed Phase I in a fasting mammal (i.e., one that is totally deprived of food) and represents the body's effort to maintain the plasma glucose concentration at its pre-fast value (Cherel et al. 1988, Belkhou et al. 1991). As glycogen from Janice's liver was being released into her blood as glucose, her adipose tissue also was releasing non-esterified fatty acids and glycerol in increased quantities into her blood. As a result, Janice began to rely more on the oxidation of fatty acids for energy while simultaneously reducing her oxidation of glucose (glucose-sparing) and her catabolism of protein (protein-sparing).

As Janice's dependency on the oxidation of fatty acids increased, she entered into a biochemical stage that is termed as Phase II in a fasting mammal (Cherel et al. 1988, Belkhou et al. 1991). During this stage, not only did her rate of fatty acid oxidation increase, but her production and use of ketone bodies also increased. Ketone bodies are a product of fatty acid catabolism which, unlike non-esterified fatty acids, can cross the blood-brain barrier and act as a fuel source to the brain. Thus, the production and use of ketone bodies was another means by which Janice reduced

her demand for glucose. As a consequence of her reduced need for glucose, less protein was required to provide precursors for gluconeogenesis. Unfortunately, however, the high production of ketone bodies and their rising concentration in Janice's blood made it difficult for her to maintain acid-base balance. To preserve acid-base balance and prevent the occurrence of metabolic acidosis (or ketosis), it was necessary for Janice to increase her production of ammonium. Thus, although the use of glucose for energy had been reduced significantly, the net breakdown of protein could not be completely stopped because protein catabolism was now required to produce the ammonium needed to preserve acid-base balance.

The fact that protein catabolism could be reduced, but not stopped, eventually proved to be fatal for Janice. While she was fully aware that her body lipid stores were diminishing by the day, she did not realize that over the months the protein from her skeletal muscle also was disappearing to provide the small amounts of glucose required by her brain for energy and the ammonium required to preserve her acid-base balance. At the time Janice made her final visit to her doctor, she still had an adequate supply of lipid available for energy, but her body protein had become severely depleted. As a result, the loss of protein from Janice's heart had exceeded a limit beyond which normal heart function could be maintained. The irregular heartbeat that Janice's doctor had detected was a ventricular arrhythmia, a sign of severe cardiovascular disease.

In many ways, the weight loss in bear X09111 appeared to occur under far more difficult conditons than faced by Janice. At the time when bear X09111 was captured with her two male cubs, she had endured 8 to 9 months of continuous fasting. In contrast, Janice had only endured 6 months on a diet that contained a very low number of calories. While fasting, bear X09111 had undergone all of post-implantational pregnancy[1] and 4 months of lactation. Janice was neither pregnant nor lactating. Finally, bear X09111 had lost 47% of her initial body weight, while Janice had lost only 39%. Nevertheless, despite the evidently greater demand on the body fuel stores of

[1] The implantation of the blastocyst in the uterine wall of polar bears is delayed for approximately 3 to 4 months following conception (Lønø 1972). Thus, mating in polar bears occurs sometime during April or May, but the development of the blastocyst is arrested until August or September, at which time the blastocyst becomes attached to the uterus and resumes its development. This process is termed delayed implantation.

bear X09111, there was nothing to suggest that the extreme loss of weight was of any ill-consequence to her health. In fact, as the records from her previous captures indicated, large fluctuations in body weight appeared to be a normal feature of her life.

There is very little known about the sequence of biochemical and physiological events that occurs in polar bears during prolonged fasting. From the results of this study, however, there are two pieces of relevant information which can be deduced. First, the response to fasting in polar bears does not conform with the scheme of biochemical responses to fasting that has been described for other mammals (Cherel et al. 1988, Belkhou et al. 1991, Castellini and Rea 1992). According to this scheme, Phase I represents a short initial period where liver glycogen stores are depleted, Phase II represents a long intermediate period where energy demands are met mostly through the oxidation of fatty acids and ketone bodies, and Phase III represents terminal starvation and occurs when 30-50% of the body protein has been depleted. Polar bears do not deplete, but instead maintain or increase, their liver glycogen stores during fasting (Chapter 6). Thus, polar bears do not show evidence for Phase I of fasting. Plasma concentrations of ketone bodies are extremely low in fasting polar bears relative to concentrations measured in other fasting mammals, and are similar to values measured in feeding polar bears (Chapter 4). Thus, polar bears do not show evidence for Phase II of fasting. Lastly, none of the 184 adult polar bears handled during this study appeared to be in a state of terminal starvation. Even the fasting bears with the lowest BCI values (< -2.0) also had low concentrations of plasma urea (< 9.0 mmol/L) and low ratios of plasma urea to plasma creatinine (U/C ratios < 12.0), which suggests these animals were able to conserve body nitrogen (Chapter 3). This is not to say, however, that some polar bears do not starve to death, but rather to say that most bears are able to fast continuously for many months duration.

The most likely explanation for why the biochemical response to fasting in polar bears does not conform to the scheme of biochemical responses to fasting described for other mammals (Cherel et al. 1988, Belkhou et al. 1991, Castellini and Rea 1992) is because, in contrast to many other mammals, polar bears subsist on a diet that is high in lipid content and low in carbohydrate content. As a consequence of this, fatty acids are the major metabolic fuel for polar bears, whether feeding or

fasting. In many other mammals (including humans), the proportions of carbohydrate and lipid in the diet are more balanced, or greater in the proportion of carbohydrate. Thus, the transition from feeding to fasting involves altering the type of metabolic fuel that is used from glucose and fatty acids during feeding to fatty acids and ketone bodies during fasting. The scheme of biochemical responses from Phase I to Phase II basically describes this transition.

Another inference that can be made from the results of this study is that lowering of the metabolic rate appears to be one important mechanism by which polar bears are able to conserve body fuel stores. Although metabolic rates were not directly measured in this study, plasma triiodothyronine concentrations and rectal temperatures were found to be lower in fasting bears captured during summer and fall than in feeding bears (Chapter 6), which suggests that metabolic rates also were lower during fasting.

7.2 Points of departure between polar bears and humans

When comparing the results from this study with the results of research that has been carried out on the need for food in humans, there are many differences between polar bears and humans (Table 7.1). Unfortunately, the significance of many of these differences remains obscure because the differences are based on concentrations of plasma constituents, and the concentrations alone do not provide information about differences in the synthesis, release, catabolism, or excretion of constituents. Nevertheless, when comparing the concentration differences between feeding and fasting for a wide range of constituents (Table 7.1), it is apparent that the energy metabolism of polar bears is remarkably constant, whereas the energy metabolism of humans is very different, between the two nutritional states. Again, this is consistent with the viewpoint that polar bears rely on fatty acids as their primary metabolic fuel whether feeding or fasting, and that humans undergo some alteration in primary fuel sources between feeding and fasting.

From the perspective of health and disease, there also are points of departure between polar bears and humans. Low sensitivity of body tissues to the effects of insulin, termed insulin resistance, is a normal feature of polar bears. In contrast, insulin resistance is a pathological feature in humans

Table 7.1 Selected biochemical constituents for which values during prolonged fasting relative to values during feeding differ between polar bears and humans.[a]

Biochemical Constituent	Fasting Value in Relation to Feeding Value	
	polar bears	humans[b]
A. Protein and amino acid metabolism		
plasma total protein concentration	0 or ↑	↓
plasma albumin concentration	0	↓
plasma globulin concentration	↑	↓
plasma essential amino acid concentration	0	↑
plasma alanine aminotransferase concentration	↓	↑
plasma alanine concentration	↑	↓
plasma creatinine concentration	↑	0
liver argininosuccinate synthetase activity	0	↓
liver argininosuccinate lyase activity	↑	↓
liver arginase activity	↑	↓
B. Lipid metabolism		
plasma total lipid concentration	0	↑
plasma non-esterified fatty acid concentration	0	↑
plasma glycerol concentration	0	↑
plasma acetoacetate concentration	0	↑
plasma β-hydroxybutyrate concentration	0	↑
plasma acyl-carnitine concentration	0 or ↓	↑
plasma free carnitine concentration	0	↓
rate of appearance of glycerol in plasma	↓	↑
C. Carbohydrate metabolism		
plasma glucose concentration	0 or ↑	↓
liver glycogen concentration	↑	↓
D. Hormone concentrations		
plasma insulin	0	↓
plasma glucagon	0	↑
plasma cortisol	0	↑

[a] ↑ indicates fasting values are greater than feeding values, ↓ indicates fasting values are less than feeding values, and 0 indicates there are no significant differences between fasting and feeding values.

[b] Comparative information for humans was extracted from Owen et al. (1969), Cahill (1970), Marliss et al. (1970), Streja et al. (1977), Hoppel and Genuth (1980), Hannaford et al. (1982), Klein et al. (1986), Clore et al. (1989), and Owen et al. (1998).

that often heralds the development of type II diabetes mellitus (Groop et al. 1989, Sugden et al. 1995, Walker 1995). It is with such points of departure, involving health in polar bears and disease in humans, that further investigation of the energy metabolism of polar bears could provide valuable comparative data for understanding, and perhaps treating, some of the metabolic diseases that occur in humans.

7.3 The study in retrospect

In the general introduction to this work, the body of the thesis is described as "an integrated collection of five parts." There were times during the course of this investigation, however, where the integration of the different studies appeared questionable, if not impossible.

At the onset of this investigation, it was anticipated that the use of stable isotope tracers would provide the bulk of the data regarding the biochemical response of polar bears to prolonged fasting. In fact, stable isotope tracers were used in the studies of protein and amino acid (^{15}N-urea and ^{13}C-urea), lipid (^{13}C-glycerol and ^{13}C-propionic acid), and carbohydrate metabolism (^{2}H-glucose). In many cases, measurement of the concentrations of different plasma constituents was made to support the results anticipated from the stable isotope studies. At the mid-point of the study, however, it became apparent that the stable isotope studies were not working and that plasma biochemistry was to provide the bulk of the data. The most obvious sign of this salvage is the large variation in sample sizes, both within (e.g., between nutritional states, between sexes) and between studies.

The stable isotope studies failed largely because most of the stable isotope tracers, with the exception of ^{13}C-glycerol, were administered at dosages that were too low to give reliable results. Nevertheless, stable isotope studies continue to offer potential for further understanding the energy metabolism of polar bears. In future, however, it will be necessary to conduct pilot studies with selected stable isotope tracers to determine effective dosages prior to carrying out full-scale metabolic studies. Such pilot studies could be conducted safely and most effectively with captive polar bears.

7.4 Future directions

Should future studies arise from this investigation, the objectives for these studies will likely be dictated as much by the research interests of the individuals involved as by any questions raised during this investigation. Nevertheless, each of the individual studies described in Chapters 3 to 6 offers questions that could provide a basis for further study. In regard to protein and amino acid metabolism (Chapter 3), the proposed model for nitrogen metabolism in the liver of fasting polar bears (Fig. 3.4) will need to be tested, and either verified or replaced with a better model. In regard to lipid metabolism (Chapter 4), the role of glycerol kinase in adipose tissue as a mechanism to finely regulate the release of fatty acids into blood will need to be determined. In regard to fatty acid composition (Chapter 5), the ability of polar bears to avoid the potentially toxic effects of large concentrations of eicosapentaenoic acid (20:5ω3) in body tissues will need to be explained. Finally, in regard to carbohydrate metabolism (Chapter 6), the hypothesis that glucose has only a minor role as an energy substrate in polar bears will need to be tested in feeding and fasting bears, possibly through the use of stable isotope tracers.

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APPENDIX A. THE SOLUBILITY OF WATER IN LIPID AND ITS EFFECT ON THE ESTIMATION OF BODY COMPOSITION IN MARINE AND TERRESTRIAL BEARS

A1. Introduction

The assessment of body composition requires knowledge of the lipid (or fat) and lean body (or non-fat) mass of an animal which, when added together, equal its total body mass. Because water is relatively insoluble in lipid, most body water occurs in the lean body mass. Thus, by measuring the body water content and the total body mass of an animal, and by knowing the relationship between its body water and body lipid content, the body composition of an animal can be estimated (Sheng and Huggins 1979, Robbins 1993). On this basis, measurement of body water content by isotope dilution has been used to estimate body composition in a variety of animals (Rumpler et al. 1987, Reilly and Fedak 1990, Farley and Robbins 1994, Hilderbrand et al. 1998).

The relationship between body water and body lipid content varies among species, and several authors have recommended that the estimation of body composition in live animals be based on data derived solely from the species under investigation (Sheng and Huggins 1979, Reilly and Fedak 1990, Gales et al. 1994). Sources of interspecific variation include differences in body conformation and in the biochemical composition of lean body mass and adipose tissue (Dawson et al. 1972, Sheng and Huggins 1979, Gales et al. 1994). The biochemical composition of adipose tissue also can vary within a species, especially with regard to water and lipid content (Worthy and Lavigne 1983, Lockyer 1991, Bowen et al. 1992, Gales et al. 1994). Adipose tissue may account for a significant proportion of total body mass in some mammals (e.g., 20 to 50% in seals, whales, and bears: McLaren 1958, Ryg et al. 1990, Lockyer 1991, Pond et al. 1992, Pond and Ramsay 1992). Thus, factors that influence the water content of adipose tissue may have important implications for estimation of body composition based on body water content and total body mass.

The insolubility of water in lipid is not absolute. A small but variable quantity of water may be mixed in lipid, depending on temperature and the carbon-chain length of the constituent fatty acids (Ralston and Hoerr 1942). The solubility of water in lipid decreases as the chain length of the constituent fatty acids increases. It follows that fatty acid composition may significantly influence the proportions of water and lipid in adipose tissue. Fatty acid composition in adipose tissue is influenced by diet (Innis and Kuhnlein 1987). Fatty acids in marine food webs tend to be longer than fatty acids in terrestrial food webs (Brockerhoff et al. 1966, Sargent 1976, Innis and Kuhnlein 1987, Rouvinen et al. 1992). Therefore, the water content (relative to the lipid content) of adipose tissue in mammals consuming food of marine origin should be less than that of mammals consuming food of terrestrial origin. To test this hypothesis, the water and lipid content of adipose tissue, the fatty acid composition of adipose tissue, and the body water and body lipid content were compared among polar bears (*Ursus maritimus*), brown bears (*U. arctos*), and black bears (*U. americanus*). Polar bears feed on marine organisms while black and brown bears feed on terrestrial organisms (Jonkel and McTaggart-Cowan 1971, Stirling and McEwan 1975, Smith 1980, Hellgren et al. 1989). These comparisons were used to determine whether or not differences in the relationship between water and lipid content between marine and terrestrial bears could significantly affect the calculation of body composition in bears by means of isotope dilution.

A2. Materials and methods

Details of the sources of polar and black bears used for the determination of adipose water (AWC) and adipose lipid content (ALC) and fatty acid composition of adipose tissue have been described previously in Cattet (1988) and Cattet (1990). Subcutaneous adipose tissue (40 to 80 g) was collected from the mid-dorsum of 25 polar bears and 25 black bears from September 1984 to November 1986. Immediately following collection, samples were sealed in air-tight plastic bags and frozen at -15°C. Duplicate subsamples (10 to 20 g) of adipose tissue from each bear were homogenized and freeze-dried to constant weight to determine AWC. The ALC was determined by extracting neutral lipids from the dried homogenate for 4 hours in a Soxhlet apparatus, with

petroleum ether as the solvent (AOAC 1980). Subsamples of frozen adipose tissue were randomly selected from ten animals of each species and submitted to the Department of Agricultural, Food and Nutritional Science, University of Alberta, for lipid separation and determination of fatty acid composition by gas chromatography (Cherian et al. 1995). Mean chain length (MCL) was calculated from the fatty acid profile of each animal using the equation:

$$MCL = \Sigma(CL \times N_{CL}) \qquad \text{(Eqn. A1)}$$

where CL is the carbon-chain length from 14 to 22, and N_{CL} is the quantity of fatty acids at a specific chain length as a proportion of total fatty acid (Holman et al. 1991).

Values for the body water (BWC) and body lipid content (BLC) of five polar bears were derived from Watts (1983), and values for six polar bears and five black bears were derived from Cattet (1988). Bears used in the former study were adult females; those used in the latter study were of both sexes, and included cubs and adults. In both studies, bears were killed either under a government management program or for subsistence. After death, bears were weighed to ± 0.5 kg and all ingesta were removed from the gastrointestinal tract. The ingesta-free carcass and viscera were then double-wrapped in plastic, weighed, and stored for 2 weeks to 6 months at -15°C. Prior to determining BWC and BLC, the carcass and viscera were re-weighed to determine water loss during storage. All tissues were then homogenized in a commercial whole-body grinder, the homogenate being passed through the grinder three times to ensure thorough mixing. Subsamples of homogenate (300 to 500 g) from each animal were collected for water and lipid analysis. The BWC was determined by weighing, freeze-drying, re-weighing subsamples, and correcting for dehydration during storage. The dried homogenate was finely ground with dry ice in a Wiley Mill and used for the lipid analysis. In the study by Watts (1983), total lipids were extracted for 18 hours using a Soxhlet apparatus with a 1:2 chloroform-methanol mixture as the solvent (Folch et al. 1957). In the study by Cattet (1988), only neutral lipids were extracted using the method described above for adipose tissue samples. Therefore, to estimate total lipid content of bears in the latter study, a

correction factor was added to the neutral lipid content based on the observation that polar lipids account for approximately 2.5% of total lipid in lean body tissues and 0.4% of total lipid in adipose tissue (Worthy and Lavigne 1983). All assays were carried out in duplicate and mean values were used in all subsequent calculations. Additional comparative data for the BWC and BLC of 13 black bears and 6 brown bears were derived from Farley and Robbins (1994).

All data were statistically analyzed using SPSS® 6.1 for Windows® (SPSS Inc., Chicago, Illinois, USA). Carbon-chain length and MCL values were compared between polar and black bears using a *t*-test for two independent samples (Zar 1996). The relationship between AWC and ALC, and between BWC and BLC, was described using simple linear regression. Slopes and intercepts for regression models were compared using small sample *t*-tests for parallelism and common intercept, respectively (Kleinbaum and Kupper 1978). Where slopes and intercepts did not differ significantly between two data sets, data were pooled to re-calculate a common regression model. For most statistical tests, significance was assigned when the probability of a Type I error was equal to or less than 0.05. When comparing carbon-chain length between polar and black bears, the interdependency of the mean carbon-chain length values in each species warranted a more conservative value for statistical significance ($p \leq 0.007$), which was calculated by using the Dunn-Šidák method (Ury 1976). All results are reported as mean ± standard deviation (SD).

A3. Results

The fatty acid composition of subcutaneous adipose tissue differed significantly between polar and black bears (Fig. A1). Myristic acid (C14:0) and fatty acids of 20 and 22 carbons were higher in polar bears, whereas fatty acids of 18 carbons predominated the profile of black bears. The relative abundance of long-chain fatty acids in polar bears was reflected by a significantly greater MCL in this species than that calculated for black bears (polar bears: 18.0 ± 0.2 versus black bears: 17.1 ± 0.1; $t = 10.6$, $p < 0.001$). By plotting the mean MCL value for each species on a solubility curve, water content in the adipose tissue (AWC) of polar bears was predicted to be approximately 71% of the AWC in black bears at equal lipid content (Fig. A2).

Table A1. Comparison of regression equations describing the relationship between body water (BWC) and body lipid content (BLC) in marine (polar) bears and terrestrial (black and brown) bears.[a]

Study	Species (*n*)	Equations	Comparison	t_{slope}	$t_{intercept}$	Common Equations	Comparison	t_{slope}	$t_{intercept}$
Farley and Robbins (1994)	black (13) brown (6)	a) 73.80 - 0.79·BLC	a) versus b)	0.83 ns	0.89 ns	e) 73.51 - 0.78·BLC	e) versus f)	2.32*	0.59 ns
Cattet (1988)	black (5)	b) 72.50 - 0.74·BLC							
Cattet (1988)	polar (6)	c) 75.64 - 0.98·BLC	c) versus d)	0.50 ns	0.27 ns	f) 74.19 - 0.88·BLC			
Watts (1983)	polar (5)	d) 74.41 - 0.87·BLC							

[a] Regression equations calculated as BWC = $\beta_0 + \beta_1$·BLC, where BWC is the predicted body water content (%), BLC is the observed body lipid content (%), β_0 is the regression intercept, and β_1 is the regression slope. Statistical significance indicated by 'ns' for not significant and * for $p < 0.05$.

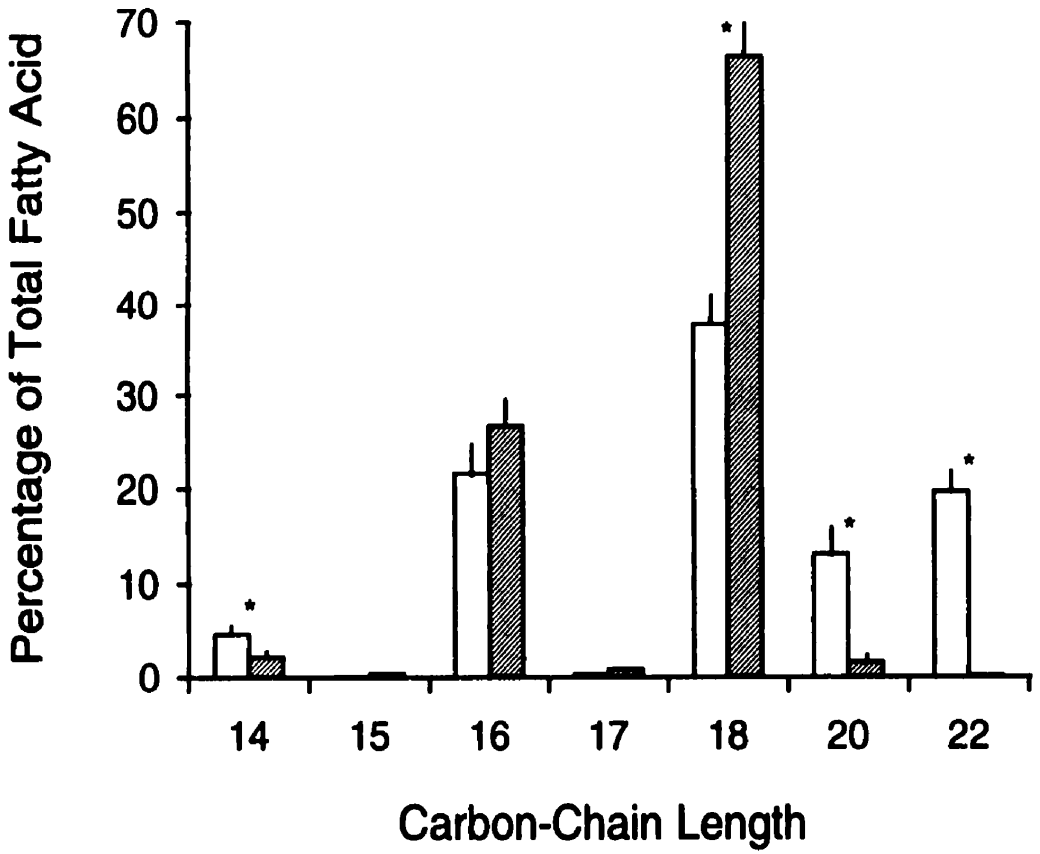

Figure A1. Fatty acid composition of subcutaneous adipose tissue in 10 polar bears and 10 black bears. Individual fatty acids are grouped within each species by fatty acid carbon-chain length. The mean proportion of fatty acid at each carbon-chain length is represented by a bar (polar - □, black - ▨) with the vertical line above indicating one standard deviation. Significant differences ($p \leq 0.007$) between species at individual carbon-chain lengths are indicated by an asterisk.

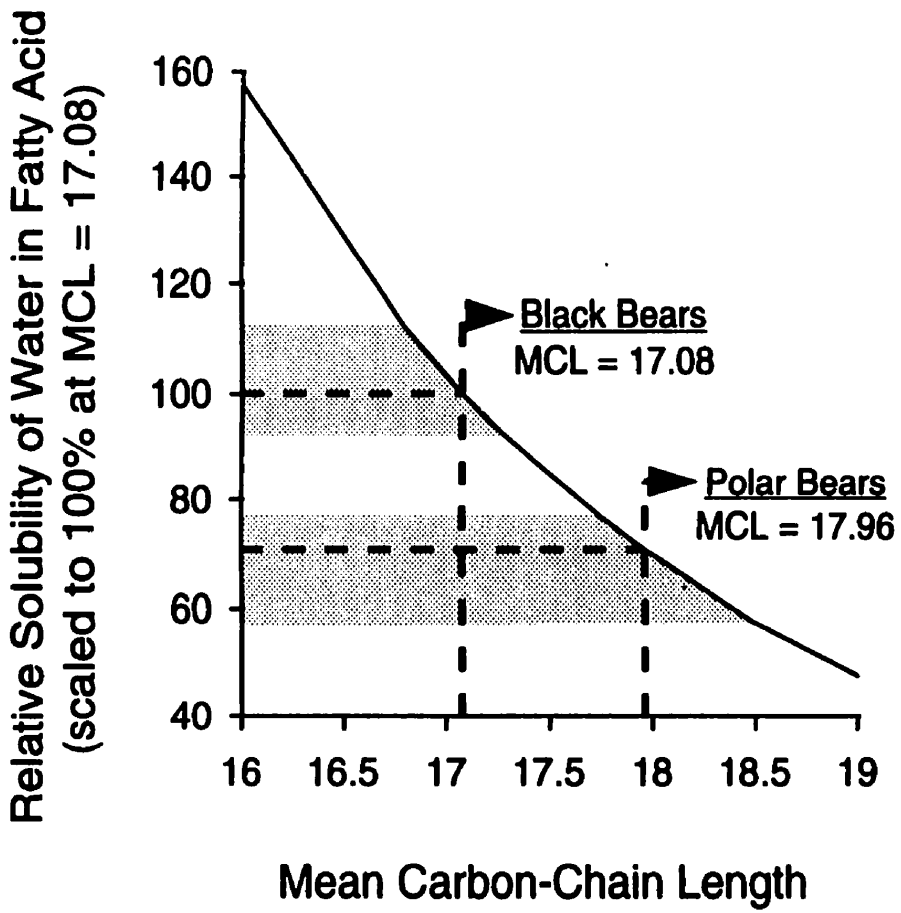

Figure A2. Comparison of relative solubility of water in fatty acid with the mean carbon-chain length (MCL) of subcutaneous adipose tissue from 10 polar bears and from 10 black bears. The solubility curve is adapted from Table II in Ralston and Hoerr (1942). Shaded areas represent the range of relative solubility values from the smallest to the largest MCL value for each individual bear within each species as labeled.

The relationship between AWC and ALC was different in polar and black bears (Fig. A3). Although there was no significant difference between species in the regression slopes ($t = 1.57$, $p > 0.05$), there was in the intercepts ($t = 2.13$, $p < 0.05$). From the regressions for each species, the AWC of polar bears was calculated to be approximately 75% of the AWC for black bears at equal lipid content.

To compare the body water (BWC) and body lipid content (BLC) data of Watts (1983) and Cattet (1988) with that of Farley and Robbins (1994), it was necessary first to make an adjustment to the values of Cattet (1988) as the combined mass of chemical components (water, protein, lipid, and ash) measured in this study accounted for only 97.9 ± 1.9% of the total body mass (TBM) of polar bears, and 98.3 ± 0.7% of the TBM of black bears. The difference between TBM and the combined mass of the chemical components was assumed to be entirely due to evaporative water loss. The proportions of water, protein, lipid, and ash in each bear were multiplied by the TBM of each bear to determine the mass for each component. The summed mass of chemical components was then subtracted from the TBM, and this difference was added to the water mass. Following this, the proportions of chemical components were re-calculated with the adjusted water mass such that the combined proportions of chemical components in each bear accounted for 100% of their TBM. Water loss was assumed to be negligible in the studies of Watts (1983) and of Farley and Robbins (1994) as the chemical components accounted for 99.3 ± 2.7% and 100.7 ± 0.6% of the TBM of bears sampled in the respective studies.

Linear regression models describing the relationship between BWC and BLC were derived separately for each set of data and compared for significant differences between slopes and between intercepts (Table A1). Data sets with equal slopes and equal intercepts were pooled to derive a common model. The slope for marine (polar) bears differed significantly from that calculated for terrestrial (black and brown) bears. The different slopes indicated that, as BLC increased within each group of bears, the difference in BWC between groups also increased (Fig. A4).

The relationships between BWC and BLC for marine and terrestrial bears were transposed [marine - BLC (%) = 82.83 − 1.11·BWC (%); terrestrial - BLC (%) = 91.93 − 1.24·BWC (%)],

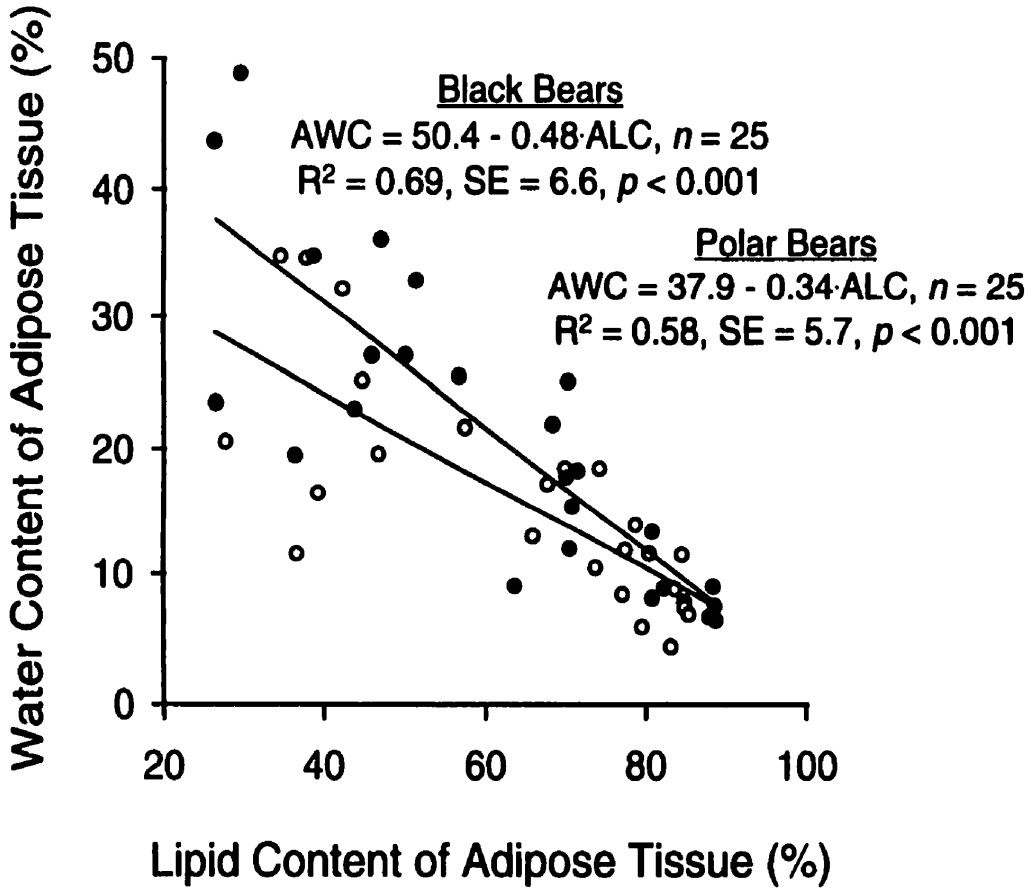

Figure A3. The relationship between the water (AWC) and lipid content (ALC) of adipose tissue in 25 polar bears (O) and 25 black bears (●). The coefficient of multiple determination is indicated by R^2 and the standard error of the predicted mean is indicated by SE.

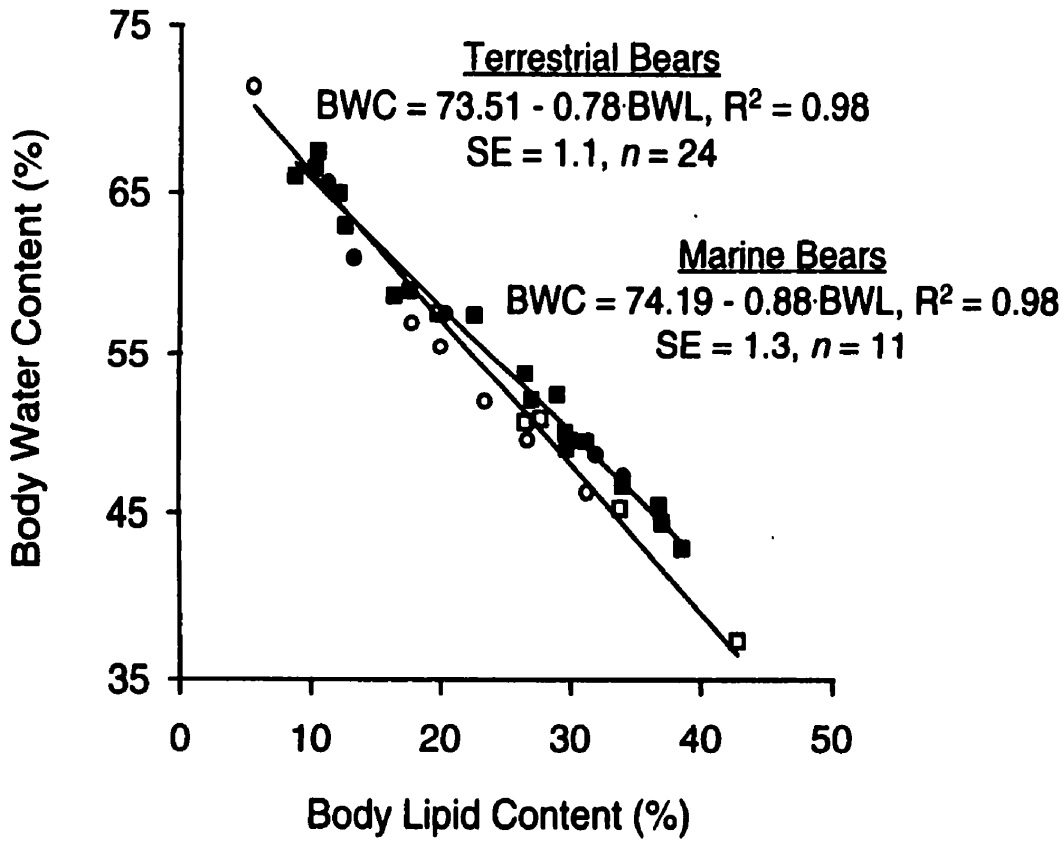

Figure A4. The relationship between the body water (BWC) and the body lipid content (BLC) in marine (polar) bears and terrestrial (black and brown) bears. Polar bear data are from Watts (1983) (n = 5; □) and Cattet (1988) (n = 6; ○). Black bear data are from Cattet (1988) (n = 5; ■) and a combination of black (n = 13) and brown bear (n = 6) data are from Farley and Robbins (1994) (●). The coefficient of multiple determination is indicated by R^2 and the standard error of the predicted mean is indicated by SE.

because measurements of BWC are frequently used to predict BLC in studies of body composition. The 95% prediction intervals for BLC in each group were then calculated and plotted on a graph to assess the amount of overlap in the prediction interval between groups of bears across a range of BWC values from 35% to 70% of TBM (Fig. A5). The prediction interval for marine bears was lower than that for terrestrial bears across most of the range of BWC values. The prediction intervals overlapped from 52% to 84% when BWC was high (≥ 52.5% of TBM), but only from 24% to 52% when BWC was low (≤ 52.5% of TBM). When BWC ≤ 38.5% of TBM, the predicted BLC value for terrestrial bears was greater than the upper limit of the 95% prediction interval for BLC in marine bears. Conversely, when BWC ≤ 40.4%, the predicted BLC value for marine bears was less than the lower limit of the 95% prediction interval for BLC in terrestrial bears.

A4. Discussion

Two observations from this analysis support the hypothesis that the water content of adipose tissue (AWC) in mammals consuming a marine diet should be lower than that in mammals consuming a terrestrial diet. First, the AWC in marine (polar) bears was significantly less than that in terrestrial (black) bears at equal lipid content (ALC) (Fig. A3). Second, the difference in AWC calculated from the regression equations for each group was similar to that predicted by the solubility of water in lipid at different MCL (Fig. A2) [i.e., the AWC of marine bears was predicted to be 75% (by regression) and 71% (by solubility curve) of the AWC of terrestrial bears at equal lipid content]. Nevertheless, the relationship between AWC and ALC varied sufficiently among individuals within both groups of bears to indicate other undetermined factors also may have influenced the relationship. Further testing of the hypothesis would require controlled experimentation with animals similar in all respects (e.g., species, sex, and age) except diet (marine- vs. terrestrial-based).

The hypothesis and supporting data have important practical implications for estimating body composition (fat mass and lean body mass) in bears based on the measurement of body water content (BWC). First, if fatty acid composition affects AWC as the results suggest, it follows that fatty acid composition also may affect BWC, especially in fat bears. In the comparison of body

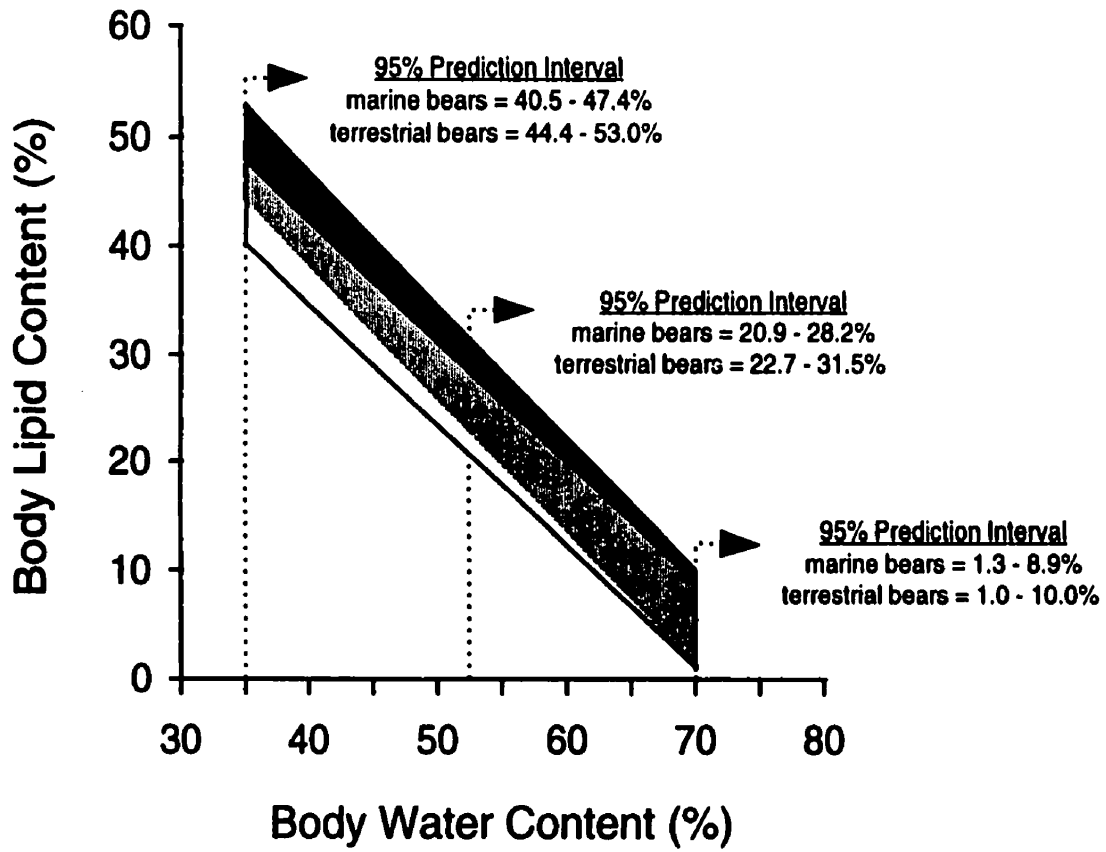

Figure A5. The 95% prediction intervals for the relationship between body lipid (BLC) and body water content (BWC) in marine (polar) bears and terrestrial (black and brown) bears. The area of overlap between the two intervals (▯) is superimposed on the individual prediction intervals for marine (▯) and terrestrial bears (■). The upper and lower BLC values for the 95% prediction interval of each group of bears are presented at BWC values of 35.0, 52.5, and 70.0% of total body mass, as indicated by the hatched lines.

fatty acid composition also may affect BWC, especially in fat bears. In the comparison of body composition data, differences in BWC between marine and terrestrial (black and brown) bears became more apparent as body lipid content (BLC) increased (Table A1 and Fig. A4). The pattern of difference was consistent with the hypothesis of this study, but any conclusions from these results must be considered tentative, since variation in other factors, including methodology, also may have had significant effect on the analysis.

A second implication is that the use of an isotope dilution model developed from the relationship between BLC and BWC measured in one species may lead to inaccurate estimates of body composition if applied to a different species, especially if there are large differences in dietary fatty acid composition between those species. This was illustrated by plotting the 95% prediction intervals for BLC in marine and terrestrial bears on a graph to assessing the amount of overlap in the prediction interval between groups (Fig. A5). When BWC was equal to 70% of TBM, the BLC values predicted by the regression equations for each group were equal (i.e., 5.13% of TBM). At BWC values lower than 70% of TBM, however, the predicted BLC values differed between groups such that the magnitude of difference became greater as the BWC value became less. Thus, when BWC was equal to 35% of TBM, the regression equation for terrestrial bears significantly over-predicted the BLC in marine bears (i.e., 48.5% of TBM predicted by equation for terrestrial bears versus the 95% prediction interval of 40.5% to 47.4% of TBM for marine bears). Conversely, the regression equation for marine bears significantly under-predicted the BLC in terrestrial bears (i.e., 44.0% of TBM predicted by equation for marine bears versus the 95% prediction interval of 44.4% to 53.0% of TBM for terrestrial bears). In the application of isotope dilution to estimate body composition in bears, this error in the prediction of BLC could be increased if the relationship between BWC as measured by freeze-drying, and BWC as predicted by isotope dilution, also differs between groups of bears.

A final implication is that the accuracy of an isotope dilution model to estimate body composition within a species may be affected by variation in the fatty acid composition among individual animals. The fatty acid composition of adipose tissue lipids (phospholipids, free fatty

acids, and triacylglycerols) in polar and black bears is highly variable among individual animals within each species (see Chapter 4), and probably reflects individual differences in diet selection and in the metabolism of dietary and stored lipids. In this regard, the large 95% prediction intervals for BLC in marine and terrestrial bears that were calculated in this study might be explained in part by differences in fatty acid composition among individual bears within each group.

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